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CONFERENCE ON SHELLFISH TOXICOLOGY,
1957

Sanitary Engineering Center
Cincinnati, Ohio

1957

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October 17, 1957

INTRODUCTION

The prevention of outbreaks of paralytic shellfish poisoning has been a problem of mutual concern to health and fishery agencies in Canada and the United States for many years. Conferences between Government and private agencies interested in this problem were held in 1943 and 1946 under sponsorship of the United States Fish and Wildlife Service and in 1955 under the sponsorship of the Public Health Service.

The objectives of the conference reported in these proceedings were: (1) Evaluate progress made since 1955 in development of a standard bioassay procedure; (2) review the maximum concentration of poison which should be permitted in market shellfish and (3) consider development of a program to allow utilization of Alaskan clam resources.

The following proceedings of the 1957 Conference on Shellfish Toxicology are based on a partial secretarial transcript and on prepared papers of the speakers. The subject matter as reported in the proceedings has been reviewed by the principal speakers. A list of the participants is included as Appendix F.

Eugene T. Jensen, Chief
Shellfish Sanitation Section
Milk and Food Program
Division of Sanitary Engineering Services

1957
CONFERENCE ON SHELLFISH TOXICOLOGY

Morning Session: Mr. John D. Faulkner, Chairman

MR. FAULKNER: I would like to introduce to you Mr. Wesley E. Gilbertson, Deputy Chief, Division of Sanitary Engineering Services, Public Health Service, who will briefly discuss the objectives of this conference.

MR. GILBERTSON: As hosts for the 1957 Conference on Shellfish Toxicology, I would like to extend a word of welcome on behalf of the Public Health Service. We appreciate very much the opportunity to get together with others having a common interest in this problem. It is a health problem with dramatic background. It excites the public interest. Certainly it is of economic significance to the shellfish industry. The solution of some of these problems is fundamental to the economic health of the shellfish industry in areas such as Alaska.

In sponsoring this meeting we hope to facilitate the exchange of information on the technical and administrative aspects of controlling paralytic shellfish poisoning. There are three major areas on which discussions have been scheduled:

First is the evaluation of progress in development of the standard bioassay procedure by seven cooperating U. S. and Canadian laboratories. We will also discuss the progress made by the Service in development of a chemical assay procedure.

Secondly, is the important question of maximum allowable concentrations of the shellfish toxin. This is something that will certainly require our best thinking.

Our third objective is a practical one, namely the development of a program which would permit utilization of Alaskan clam resources. Thus, we hope to move from the evaluation of research to its practical application in field control practices and procedures.

Again in opening this meeting I want to assure you of the continued interest of the Public Health Service in this problem, and to offer our very best wishes for a successful meeting. I know you will bear in mind the public health importance as well as the economic aspects of your discussion.

MR. FAULKNER: Thank you, Mr. Gilbertson. The first item on our agenda for this morning session is the paper on "Experience with the Reference Standard in the Bioassay for Paralytic Shellfish Poison", which will be presented by Dr. E. J. Schantz, Army Chemical Corps.

Note: Dr. Schantz presented a prepared paper, a copy of which is included as Appendix A of this report.

MR. McFARREN: I think there is some confusion about the CF value and that we should discuss its use. This value should not change appreciably unless there is a change in the source of mice or in the procedure used; however, it should be rechecked periodically with 5 mice. If the resultant value is plus or minus 20% of the original CF value there is no need to recompute the CF value. At the Sanitary Engineering Center we have used the same CF value for two years. It has never differed from the original by more than 20%. However, most of our results, as just presented by Dr. Schantz, were obtained in a period of a few weeks.^{1/}

DR. SCHANTZ: Our experience over a longer period of time indicates that some readjustments may be necessary.

MR. PAGE: Wouldn't it be more convenient to set up a conversion table in micrograms rather than mouse units and thereby eliminate the conversion factor?

DR. SCHANTZ: The last conference expressed the desirability of using micrograms instead of mouse units to give the results of assays.^{2/} I think we should report in micrograms since this makes it possible to compare the data from different laboratories. However, the CF value is still needed to make the conversion from mouse units. Also, most of our older data is given in mouse units.

MR. JENSEN: I think that Mr. Page was suggesting that each laboratory make a dose response table in micrograms based on their own data.

MR. McFARREN: It is possible but each laboratory will have to construct their own table by using the CF value obtained on their mice. Such a table will have to be changed whenever the CF value is changed. A dose response table for a CF value of 0.18 is presented in our bioassay paper.

DR. SCHANTZ: It is probably easier to use the Sommer table and to change to micrograms by using the conversion factor. However, results should be expressed in terms of micrograms.

^{1/} The use of the CF value is described in "Interim Plan for Standardizing the Bioassay of Paralytic Shellfish Poison by Use of a Reference Standard", copies of which are obtainable from the Public Health Service.

^{2/} "Conference on Shellfish Toxicology, May 26, 1955".

MR. HAGAN: Does sex have anything to do with mouse response to the poison?

MR. McFARREN: At the Sanitary Engineering Center we have checked with 40 to 50 mice, but did not find a statistically significant difference. This is not in accord with the findings of Pugsley.

DR. SCHANTZ: We have to recognize that some people believe there is a significant difference. However, if we picked mice at random sex difference should be eliminated and we should not have to be concerned.

DR. NELSON: The weight limitations might make it difficult to select at random. For example, it would be quite easy to have a 7 to 3 ratio.

MR. McFARREN: I believe that the weight of the mouse is the most important factor in the test. The mice should preferably be in the 19-21 gram weight group. A correction factor can be used for mice outside of this range; however, this has limitations. Mice weighing more than 23 grams should never be used.

DR. HALSTEAD: What about genetic strain?

DR. SCHANTZ: In the first assays carried on with the Fish and Wildlife Service back in 1949 we found that different strains of mice gave significantly different results. The only practical way to get around this variation is through the use of the purified standard and the CF value.

MR. D'AGUANNO: Then there is a strain difference?

DR. SCHANTZ: We know there is a difference. However, the total variability is included in the conversion factor.

DR. TENNANT: Much would seem to depend on the source of mice. Do the other laboratories raise their own mice or do they buy them? You might have to repeat the CF determination on each assay if you were not sure of the genetic strain. Also it might be impossible to get a random sex distribution. We buy our mice from a supplier. They are sorted by sex when they are shipped to us. Therefore, we use only females in the test. This is an arbitrary decision on our part.

DR. HALSTEAD: We made about 14,000 bioassays with mice in testing for other fish toxins. We found considerable difference in results with different genetic strains. You do not get consistent results unless you use the same strain. If you are going to standardize this assay procedure I would suggest that a specific strain be selected and specified in your assay procedure.

DR. SCHANTZ: The laboratories are scattered all over the United States and Canada. It would be difficult for them to get mice of the same strain.

DR. HALSTEAD: It is very important. For example, in tumor screening it has also been found necessary to base findings on a specific strain. I don't think you can set up a standard without specifying the genetic strain.

DR. LEWIS: This is the very purpose of the purified reference standard and the related conversion factor. It eliminates the difference in results that are attributable to strain of mice or other factors which may be peculiar to a particular laboratory.

DR. SCHANTZ: The only practical solution is to get the best mice you can and then to use a standard preparation for correcting the variations. You would jeopardize the practicability of the assay procedure by any other procedure.

DR. NILSON: At the Fish and Wildlife Service we have made some assays with 3 or 4 strains, different sizes and sexes, and have determined death by time of last reflex and by time of last breath. Results varied by as much as 40%. On any assay you should apparently use only one strain and one weight of mice.

DR. SCHANTZ: I had the opportunity to work with Dr. Sommer on this in the early days. We couldn't always get the same mice and we found great variation from source to source.

MR. MEYERS: The same is true in Alaska. We now raise our own mice.

DR. SCHANTZ: In Ketchikan we had to have mice flown in from Seattle. They arrived in poor condition and we had to get them back in good shape before we could use them. By that time they were too heavy. However, we still had to use them. We have found that variations between laboratories may be as much as 50% due to variations in the mice. This can be narrowed down to about 15% by using the reference standard and a CF value.

DR. NILSON: What is the effect of salt on the assay?

DR. SCHANTZ: There is a "salt effect". We have investigated this, using non-toxic clams to which we added known amounts of purified poison. In the presence of one percent salt our recoveries were reduced as much as 50%. Less than 0.1% salt did not reduce the recovery appreciably. It is an important point to consider in the assay of canned clams to which salt has been added. Probably some adjustment will have to be made in our assay procedure to compensate for this. Any sample of clams sufficiently high in toxicity to allow a dilution of salt to less than 0.1% should not show a salt effect. The collaborative studies that are now being started should give us more information on this point.

DR. HORTON: You indicated that variations of plus or minus 20% in assays were acceptable. Can this be obtained with only three mice?

MR. McFARREN: Yes, however, in order to arrive at the dilution which will cause the mice to die within 5 to 7 minutes, you actually may have to use more than three mice. At death times less than 5 minutes, the response curve is very steep and at more than 7 minutes, it is very flat. Three mice are a sufficient number on which to base calculations of the toxicity provided all three mice are injected with the dilution which causes the mice to die with a median death time between five and seven minutes.

DR. TENNANT: How do you compute the median death time? What do you do about survivors?

DR. SCHANTZ: The survivors are included in the computation of the median. Simply make an array and take the median value.

DR. TENNANT: Is that statistically sound?

DR. SCHANTZ: Yes, we have discussed this with statisticians at the Sanitary Engineering Center and at the Chemical Corps.

DR. LEWIS: It isn't an ideal situation but it is certainly better to take the survivors into account than to ignore them.

MR. McFARREN: The use of three animals gives you results that are within the plus or minus 20% range. If 10 mice are used this can be reduced to plus or minus 12%. Use of an LD 50 would require even more animals. We doubt that the small relative gain would justify the added cost of using 10 or more mice for each determination.

MR. FAULKNER: If there are no more questions we will have a short break and will then proceed with the next item on our agenda, discussion of the need for future collaborative studies of assay procedures and development of a tentative standard bioassay procedure which will be presented by Dr. Keith Lewis, Chief, Milk and Food Research, Robert A. Taft Sanitary Engineering Center.

DR. LEWIS: I would like to point out once more that prior collaborative studies have indicated variations in the results obtained by various laboratories. There are factors in the bioassay that must be controlled if we are to do a fair and reasonable job in keeping toxic shellfish out of the markets without doing undue damage to the shellfish industry.

The recent studies with purified shellfish poison gives us reason to hope that with the additional work proposed we can analyze shellfish in different laboratories and, by use of a standardized procedure, come up with comparable results.

The chemical assay has not been tried by anyone outside of our own laboratory as far as we know. We have completed the development of the procedure and are now working on various improvements in the basic technique. It is not a simple field test as we had hoped it might be. However, it may be more accurate than the bioassay and may give higher values in the lower ranges of toxicity. It is not affected by salt.

The proposed collaborative study will attempt to determine whether the bioassay and chemical procedures are feasible, and the extent of variations which will result when they are used by different laboratories. We believe the opportunity is here to make a real advance in the accuracy of the bioassay for paralytic shellfish poison. In time the chemical assay may replace the bioassay.

A collaborative study is, as many of you know, now being initiated under the auspices of the Association of Official Agriculture Chemists. Mr. Earl McFarren has been appointed as associate referee for the study. A plan for the collaborative study has already been presented to the eleven interested laboratories.

Briefly, this is what we hope to do in the collaborative study:

1. Study the standardized bioassay procedure using purified shellfish poison as the reference standard and establish a CF value for each participating laboratory. Most of the laboratories have already completed this phase.
2. Make comparative bioassays of non-poisonous clams to which will have been added known amounts of purified shellfish poison. Also comparative bioassays will be made on naturally toxic clams. The CF factor established in phase 1 will be used.
3. Standardize the chemical assay against the bioassay using both clams with purified poison added and naturally poisonous clams.

The laboratories which have agreed to participate in this study are:

1. Fishery Products Laboratory, Ketchikan, Alaska.
2. Arctic Health Research Center, Anchorage, Alaska.
3. Food & Drug Laboratory, Department of National Health and Welfare, Ottawa, Ontario, Canada.
4. Food and Drug Administration Laboratory, Department of National Health and Welfare, Ottawa, Ontario, Canada.
5. Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Ontario, Canada.
6. Chemistry Branch, Fort Detrick, Frederick, Md.
7. State Department of Public Health, Berkeley, California
8. Chemical Research Division, Army Chemical Center, Md.
9. Food and Drug Laboratory, Dept. of National Health and Welfare, Vancouver, British Columbia, Canada

10. Pharmacology Division, Food and Drug Administration,
Department of Health, Education, and Welfare,
Washington, D. C.
11. Sanitary Engineering Center, Cincinnati, Ohio.

Mr. McFarren is now seeking someone who can furnish us with 3,000 gram samples of shellfish meats. If possible, we would like to get lots of shellfish that have three different levels of toxicity. We would also like more than one species of clam. Clam samples for the collaborative assay will be ground, preserved in 25% methanol acidified with hydrochloric acid and mailed out in 100 gram aliquots in polyethylene bottles. This is certainly a far cry from fresh clams; however, we must have uniform samples for all of the laboratories.

DR. LEHMAN: Are you sure the polyethylene bottles are inert? We have had some difficulties with material leaching out of the plastic.

MR. McFARREN: We haven't really checked this carefully.

DR. LEHMAN: You might want to investigate this. There is a silicone lined polyethylene bottle that is used for cosmetics.

MR. UDELL: There should be no real problem in shipping clams to Cincinnati.

DR. LEWIS: There is always the problem of unexpected delays in shipment and of refrigeration during shipment.

MR. UDELL: Our dealers routinely ship oysters to the West Coast. I am sure that we could supply you with the non-toxic clams.

DR. NILSON: We have had trouble shipping fresh clams. Why not ship them in the shell? You could make multiple shipments to insure that at least one of them would get there in satisfactory condition.

MR. BOND: We can ship toxic clams but we can only be sure of getting them during the summer. We are now down to about 500 mouse units in our most toxic areas. I would also suggest they be shipped in the shell.

MR. McFARREN: We could shuck them and store them in the freezer until needed. How many clams would it take to make a 3,000 gram sample?

DR. NILSON: A bushel of soft clams would give you plenty of meats.

DR. LEWIS: We would certainly appreciate having your help in obtaining the needed supply of clams. Perhaps anyone who could help would get in touch with Mr. McFarren.

MR. FAULKNER: If there are no further questions which the group would like to discuss with Dr. Lewis, I would like to present Dr. Bruce Halstead, who will discuss the topic of poisoning by fin fish. I am sure that most of you are acquainted with Dr. Halstead's extensive research work in this field.

Dr. Halstead presented a prepared paper, a copy of which is included as Appendix B of this report. A short color film on fish poison was also shown by Dr. Halstead.

Afternoon Meeting: Dr. Keith Lewis, Chairman

DR. LEWIS: The first item on our agenda this afternoon is a discussion of the chemical assay for paralytic shellfish poison by Mr. Earl McFarren, Milk and Food Research, Sanitary Engineering Center.

Mr. McFarren presented a prepared paper, a copy of which is included as Appendix C of this report.

DR. LEWIS: Are there any questions on Mr. McFarren's paper?

MR. MEYERS: An interference factor of 87 micrograms was mentioned. What does this represent? What is it in terms of percentage error?

MR. McFARREN: The Jaffe test is not specific for the poison as many other substances also react in this test. Some of these substances are removed by the ion exchange column treatment but not all. An interfering substance (s) naturally present in clams is not removed. The amount of this interfering substance (s) varies with the age or state of decomposition of the product. However, the procedure developed for the chemical assay of the poison has been designed to correct for this interfering material, regardless of the amount present. This interfering substance (s) seems to be most like histamine in its adsorption characteristics on the ion exchange column and in its reaction when the color is developed in the Jaffe test at two different temperatures.

It should be pointed out, however, that several of the chemicals used in the test may contain impurities which will interfere in the test. This source of interference can be corrected only by using purified reagents in the test. For example, ethanol has been suggested as

a preservative in the shipment of clams for study, but it has been found that ethanol reacts in the Jaffe test when the solution is heated to develop the color. Methanol is a better preservative for this purpose, but some grades of methanol may contain relatively large amounts of aldehydes and ketones which interfere in the test. Also, the ether used in the test must be specially purified to remove some of these same substances before being used to extract the trichloroacetic acid from the column eluate.

DR. SCHANTZ: We must keep in mind that the tests are given by a variety of substances. The problem is to separate interfering substance from the poison. The Jaffe and Benedict Behre tests both measure a characteristic related to toxicity and are specific in this respect.

MR. SASS: Impurities in the alcohol can be removed by a silver-nitrate-caustic treatment. This could be specified in the method.

DR. LEWIS: I am sure we will find ways to improve this procedure as we become acquainted with it. Its a new procedure and it has not been tried out by anyone else. We hope other labs will take it up.

MR. SASS: In our studies we are wondering how far we must go in following the procedures for the collaborative study, particularly with the chemical assay.

MR. McFARREN: I believe we should follow exactly the bioassay procedure since we have had a great deal of experience with it. In the chemical assay, if difficulties are encountered, some modifications may be necessary.

DR. LEWIS: However, in the collaborative study we want comparable results. All laboratories will have to follow the same procedure to do this. An article on the bioassay and an article on the chemical assay are going to be prepared and published in the Journal of Association of Official Agriculture Chemists. In fact the manuscripts have already been cleared for publication.

DR. NILSON: Have you tried the assay on scallops?

MR. McFARREN: No, we have had little experience with mussels or scallops. Most of our work has been with clams. Frankly, I don't know what the problems would be with scallops.

MR. SASS: We have forms for reporting bioassay but nothing on chemical assay. Do you have such a form?

MR. McFARREN: No, we haven't any chemical assay forms as yet. However, a detailed chemical assay procedure will be drawn up and report forms provided similar to the "Interim Plan" which you are using in the collaborative study on the bioassay.

DR. HALSTEAD: Would it be possible to send us a copy of the two publications that are coming out?

DR. LEWIS: We will be glad to send out reprints as soon as they are available. The two papers should be published in February--about the same time as these proceedings.

The next subject on our agenda is a discussion of 'Maximum Allowable Concentration of Paralytic Shellfish Poison in Fresh and Canned Shellfish' by Mr. R. M. Bond, Officer in Charge, Fish Inspection Laboratory at St. Andrews, Canadian Department of Fisheries.

Mr. Bond presented a prepared paper, a copy of which is included as Appendix D of this report.

DR. HALSTEAD: How did you warn the public that the clams in the area were not safe?

MR. BOND: Warning signs are posted. These signs read: "WARNING. The clams in this area are affected by paralytic shellfish poison and are unfit for human consumption unless treated by the canning process".

Incidentally we have found that clams scoring about 1,000 mouse units can be successfully canned and will produce a consistently poison-free product. We have also experimentally canned some red mussels with an initial score of about 12,600. The residual toxicity a week later was 650-750 mu. Approximately two weeks after canning the toxicity averaged 1,000; three weeks later it was 1,400 and 2 months later 960.

Mussels with an initial toxicity of 5,800 were canned. Five days after canning they scored 320 mu. (July 15). They were then re-tested July 26 and showed 320 mu. On August 20 they scored 320 and on October 4, 310 mu.

It seems that storage is not quite as effective as we formerly thought in reducing toxicity. We now feel that clams with scores over 1,000 mu. are not acceptable for canning.

MR. MCFARREN: How do you sample canned clams?

DR. TENNANT: We just use the liquor. The liquor and meats should be in equilibrium.

MR. BOND: This was investigated in 1945. Present results indicate that equilibrium may not be reached until a month after canning.

MR. McFARREN: Our procedure is to prepare a liquid-meat extract on the basis of liquid/solid ratio in the can.

DR. HALSTEAD: In regard to the pre-investigation of an area that is about to be toxic--do you make a plankton study?

MR. BOND: No. However, we think that this is a missing link; while we are certain that a dinoflagellate is the source of the poison, plankton studies are not used as a control measure.

DR. HALSTEAD: California also has the problem. We now wait until shellfish becomes intoxicated before an area is closed. A planktonic organism is responsible for the toxin. Perhaps toxicity could be predicted by fluctuation in plankton.

MR. BOND: We feel that this should be done. When clams were toxic we made a few plankton tows and found Gonyaulax tamarensis was present.

DR. HALSTEAD: If you watch the population fluctuation of dinoflagellates closely you can predict when toxic shellfish are most likely to occur.

DR. SCHANTZ: That is the procedure Dr. Sommer used in California.

MR. BOND: We have used a key station type of control with great success. For example, it was found that Head Harbour, Campobello Island, produced toxicity approximately two weeks before it occurred on the mainland. This worked up until last year. Now we may have to revise our thinking on that point.

DR. HALSTEAD: I think a completely safe public health technique probably must be based on an analysis of the dinoflagellate population.

MR. BOND: Dr. Medcof and I believe this should be done. We need some basic research on the dinoflagellate which we could relate directly to temperature. The temperature would then give us a warning.

DR. HALSTEAD: The British have just isolated a new toxic Gymnodinium which seems to operate by a process of delonization of nerve membranes. Gonyaulax poison affects the respiratory and vasomotor centers and neuromuscular functions. The research on this poison has been done within the last two years at the Marine Biological Laboratory, Plymouth, England.

MR. D'AGUANNO: What are the Canadian tolerances for poison in canned shellfish?

MR. BOND: There is no tolerance. They must be free of poison.

DR. LEWIS: Our last topic on the agenda, problems in utilization of clam resources of Alaska due to the presence of paralytic shellfish poison will be presented by Mr. W. B. Page of the Public Health Service Arctic Health Research Center.

Mr. Page presented a prepared paper, a copy of which is included as Appendix E of this report.

DR. SCHANTZ: There doesn't seem to be much evidence that Gonyaulax is the source of the toxicity in butter clams. However, the isolated poison from mussels, which we know is from Gonyaulax, and the poison from the clams, which we are in doubt about, are identical as far as we know. This supports the idea it might be from Gonyaulax. In fact, at times you do find a few organisms in the (Alaskan) waters. But the thing that always puzzled us was that clam beds only a half mile apart would vary greatly in toxicity. The water seemed to be quite thoroughly mixed by the tide and it was difficult to understand why one bed would be toxic and the other not when they were both washed by the same water.

MR. GLUDE: How rapidly do shellfish get rid of the poison? Has anyone held toxic clams and tested them over a period of time?

MR. MAGNUSSEN: Yes, we moved toxic butter clam to beds where clams were non-toxic. In one year the toxicity dropped to about 1/3 the original level.

MR. GLUDE: In the Chesapeake Bay, we are interested in a red discoloration in clams and oysters. We don't know if this is a food which is ingested and absorbed quite rapidly or if it continues over a long period of time. We do plan to hold clams for various times to see if they become clear again.

MR. BOND: Dr. Kerswill has done some similar work at St. Andrews Biological Station.

DR. LEWIS: Do clams in Alaskan waters tend to keep the toxin longer than in the Canadian waters?

MR. BOND: Well, prior to 1950, we had toxicity the year around in some areas. Ordinarily toxicity in soft-shell clams goes up fast and comes down again in a very short time.

DR. SCHANTZ: Alaskan butter clams stay toxic all year because much of the toxin is in the siphon. In the soft clams it is in the viscera.

MR. MAGNUSSEN: Mr. Meyers, were razor clams tested for toxicity?

MR. MEYERS: Yes, but I do not know the distribution of the poison in the clams.

MR. MAGNUSSON: When I was in Alaska we failed to find toxin in the razor clams. Did you use the entire clam?

MR. MEYERS: Yes, only the siphon tip was removed.

DR. LEWIS: If we have no further discussion on the Alaska situation, I would suggest we turn the meeting over to Mr. Jensen to summarize.

MR. JENSEN: When the meeting was opened three major objectives were outlined by Mr. Gilbertson, two of which related to the collaborative study of the bioassay and the colorimetric test. We have had a thorough discussion of these assay procedures and I think there is little more to be said regarding the method. However, going back to the 1954 conference on shellfish toxicity, it was recommended that results of assays be expressed in micrograms rather than mouse units. Does the group want to repeat that recommendation or wait the development of AOAC method? Would it be premature to make such recommendation?

MR. McFARREN: We cannot hope to be reporting our results in equivalent units until we have adopted the concept embodied in the use of a conversion factor to convert mouse units to micrograms of poison.

DR. LEWIS: Certainly there is no question of the usefulness of the CF and the microgram value when widely separated laboratories are involved.

DR. NILSON: Are we sure that there is only one toxin, particularly in products from Alaska? If not, this recommendation should not be made until we are sure.

MR. JENSEN: The purpose of the CF value is to facilitate the comparison of the results obtained by the various laboratories.

DR. NILSON: Still we must be sure there is only a single toxin.

DR. SCHANTZ: We feel quite certain that there is only one toxin. If there are more than that they must be closely related chemically. Perhaps bioassays would also be identical.

MR. MAGNUSSON: Didn't Dr. Sommer feel that there were three or four toxins in mussels?

DR. SCHANTZ: At first, but he did not hold to the idea.

MR. JENSEN: It is then my understanding that the conference will again recommend the use of the standardized test procedure as outlined in the Interim Plan for Standardizing the Bioassay of Paralytic Shellfish Poison by Use of a Reference Standard. It will be further understood that three mice will be considered adequate for routine testing if properly used.

MR. BOND: It is now Canadian practice to use three mice.

DR. TENNANT: However, we extended the number of mice to 10 in testing important extracts collected during the 1957 outbreak in New Brunswick.

MR. JENSEN: The last objective which was outlined this morning related to the Alaska shellfish. The Public Health Service is not now directly concerned with the shellfish industry in Alaska because a great proportion of the product is heat treated and canned. However, there has been some shipments of fresh or frozen clams to the West Coast and the Territory has indicated an interest in Service endorsement of their shellfish sanitation program. The big problem there seems to be control of toxicity and to establish an administrative tolerance. We have thus far used the tolerance limit adopted by Food and Drug Administration about ten years ago. The basis for this value was described at the last conference on paralytic shellfish poison in 1955. Are there any ideas if this standard is still applicable or if we should think about a lower tolerance level, as Mr. Bond has suggested for the East Coast?

MR. HAGAN: We will have to discuss this point further before we make up our mind on it.

MR. BOND: Your acceptance level is higher. The acceptable level in Canada for raw clams is 400 or less and none in canned clams.

MR. HAGAN: Our standards are: For whole clams, fresh, frozen or canned, the average toxicity shall not exceed 400 mu. per 100 grams and no individual package shall exceed 2,000 mu. With minced clams, the average toxicity must be less than 2,000 mu. per 100 grams and no individual package shall assay materially above 2,000 mu.

MR. JENSEN: I believe the purpose of this type of standard is to recognize variation in testing procedure.

DR. TENNANT: Canada would not allow a pack at the 2,000 level. We accept absolutely no toxicity in canned clams.

MR. BOND: Our beaches are closed to shucking when the score is 400 or above. Up to 1,000 they are open for canning. We had an unusually early rise this year. Up to that time we had no idea of closing the beaches entirely.

MR. JENSEN: Mr. Bond has indicated there is an overall trend toward colder water?

MR. BOND: Yes, we came across this prediction last year in an article published by the Atlantic Oceanographic Group at St. Andrews (Canadian Fisherman, Vol. 43, No. 9, 1956). Our past experience indicates an inverse relationship of toxicity to winter water temperatures. If the temperature rises, the toxicity decreases, and if the temperature

decreases, the toxicity increases. If the long range prediction for lower water temperatures is correct, we can expect a period of high toxicities which will be at least as high, if not higher, than in any previous experience. On the basis of the statement by the Oceanographic Group we predicted toxicity would be higher than last year. The prediction was verified.

DR. LEWIS: Would it extend toxicity further down the Atlantic Coast?

MR. BOND: That would be difficult to say.

MR. JENSEN: Certainly the matter will have to be given more thought in New England than in the past. However, I would like to continue the discussion of the Alaskan situation a little more. A few years ago B. C. Packers was quite interested in shipping Alaskan clams to Prince Rupert, B. C. for canning. For a while we hoped a control program similar to the one used in the Maritime Provinces might be developed for Alaska. However, further discussion lead to the recommendation that if clams were sold fresh, there would have to be an official sample for each lot that left the Territory. We felt we should have a high degree of confidence in each sample. Should this principle also be applied to razor clams from Alaska? Are there any ideas from the group on this?

MR. MAGNUSSON: Are they still canning razor clams?

_____: They are subject to testing after canning.

MR. JENSEN: We are not too much concerned with this in Public Health Service if clams are processed. This is adequately handled by existing standards. However, fresh clams almost have to be from a certified source to be sold without difficulty. Are there any suggestions on this?

MR. D'AGUANNO: Could you put them in clean water until they cleaned up? Similar to purification.

MR. BOND: No, it couldn't be done economically.

DR. SCHANTZ: It would take too long. Mr. Magnusson mentioned a year for 2/3 of the poison.

MR. JENSEN: Apparently there is no simple solution to the Alaskan clam problem at this time.

MR. PAGE: What is the Canadian tolerance in canned clams?

MR. BOND: No toxicity.

DR. LEVIE: I want to make one observation. We have concentrated our work on assay procedures. However, there is a great need for ecological studies directed at field control. Ultimately, we must know much more about this area to develop good controls.

MR. GLUDE: That is certainly true. Methods of assay and public-health aspects are a lot better known than are the biological problems. We should devote time to the biological studies.

MR. FAULKNER: Are there any additional questions which you would like to have discussed. If not, I would like to express our appreciation to everyone for taking the time to come to Washington to participate in this meeting. Paralytic shellfish poison is an important Public Health Service problem. It appears that we are certainly progressing with the assay procedures, and it is hoped that before too long we will be able to develop a positive approach for handling the Alaskan problem. The meeting is now adjourned.

A P P E N D I X A

**PURIFIED SHELLFISH POISON FOR
BIOASSAY STANDARDIZATION**

By

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PURIFIED SHELLFISH POISON FOR BIOASSAY STANDARDIZATION

Poisoning due to the ingestion of toxic shellfish has been a problem of mutual concern to health and fishery authorities in Canada and the United States for many years, and has been recognized for over a century as a clinical entity (5). Most of the reported cases of shellfish poisoning have occurred along the central California coast, (4) but a sprinkling of extensive outbreaks have been reported from Alaska (4) to southern California. More recently cases of poisoning due to mussels (3) have been reported in the area of the Bay of Fundy, Nova Scotia and New Brunswick, and to the soft-shell clam (10) on the south shore of the St. Lawrence River between Metis Beach and Baie des Capucins, Quebec.

The poison found in California ocean mussels and in some clams originates in a unicellular microscopic organism of the ocean, the dinoflagellate, Gonyaulax catenella (9). This organism is free swimming and serves as food for many animals of the sea, including the bivalves. A concentration of 20 to 40 thousand toxic organisms per ml imparts a red rust color to the water, and under these conditions mussels become extremely toxic. Even much lower concentrations of the organism (1000 or less) that impart no visible color to the water may cause the toxicity of mussels to reach dangerous levels for human consumption within a few days. About 90 percent of the poison found in mussels is localized in the digestive gland (9) and apparently is not harmful to the shellfish. As the organisms disappear from the water the poison in the digestive gland is almost completely destroyed or excreted within one to three weeks.

The primary source of the poison found in the Alaska butter clam is not definitely known. In contrast to mussels these clams may remain toxic for years with only slight seasonal variations. About 70 percent of the poison is localized in the siphon (2). When butter clams are removed from the environment where they became toxic, a year or more is required before the amount of poison is appreciably decreased.

In 1943 the Public Health Service, the Fish and Wildlife Service, and the Food and Drug Administration adopted a tentative procedure (11) for the assay of poisonous shellfish and recommended certain tolerances to be used as a guide in closing shellfish digging operations. Upon the basis of these procedures and recommendations, early in 1946 the Food and Drug Administration withheld shipments of canned Alaskan butter clams (2), Sexidonus giganteus, because of possible hazard to humans. Under these circumstances, the Alaskan packers ceased operations until the situation could be clarified. The Fishery Products Research Laboratory, Fish and Wildlife Service, Ketchikan, Alaska, undertook extensive studies of the problem in that area (2) and in

1949 sponsored a collaborative study(12) on the assay of toxic clams. This study showed that the results of one laboratory may differ from another by as much as 60 to 70 percent and demonstrated a need for a standardization of the bioassay.

In May, 1955 the Public Health Service sponsored a Canadian-United States Conference on Shellfish Toxicology(12) to discuss improvements in the assay procedure. At that time, the isolation of the poison in pure form by Schantz and associates (7, 6) was announced, and the suggestion was made that the purified poison be used as a reference standard for the bioassay. The poisons from the Alaska butter clams and California ocean mussels appear to be identical in chemical, physical and biological properties. The dihydrochloride salts are white solids soluble only in water, methanol and to some extent in ethanol. They have a toxicity of about 5.5×10^6 mouse units per grams, possess no absorption in the ultra violet and visible range, and have a specific optical rotation of $+130^\circ$. None of the above properties have been found to change on storing the poison in the dried state or in acid solution during a period of two years and it is believed that the poison is sufficiently stable to serve as a reliable reference standard. The Army Chemical Corps has offered a limited quantity of the purified poison to the Public Health Service for standardization and research purposes.

On the basis of this and other information presented at the 1955 Conference, the conferees agreed that the purified poison should be used as a tentative reference standard to obtain more uniform bioassays and the results of future assays should be reported in terms of the weight of the poison. Accordingly, a study has been made of the requirements for preparation, distribution, and use of the standard. An interim plan for utilizing purified poison to standardize the bioassay has been drawn up by the Public Health Service (13) and may be obtained on request from the Shellfish Sanitation Section, Washington 25, D. C.

The studies presented herein indicate the basis for establishing certain standard conditions under which to determine the micrograms of purified poison equivalent to one mouse unit. They also demonstrate the usefulness of the reference standard in assaying poisonous clams. Standardization of the bioassay was an essential step in the development of a chemical assay for shellfish poison. The chemical determination of shellfish poison will be the subject of a subsequent paper.

EXPERIMENTAL PROCEDURES

Preparation of standard solution - Five to ten milligrams of the purified crystalline poison were weighed on a semi-micro balance to the nearest one-hundredth of a milligram. Because the poison is hygroscopic, it was necessary to dry it to constant weight in a vacuum desiccator over phosphorous pentoxide and weigh it in a dry atmosphere.

For every milligram of the weighed amount of poison, 10 ml. of a solution containing 0.1 N hydrochloric acid and 20 per cent ethanol were added. The acid was used to insure stability of the poison and the alcohol to inhibit the growth of fungi. This solution then contained 100 micrograms of poison per ml. The solution was mixed well and stored in the refrigerator for 24 hours in order to allow it to reach equilibrium before using it for assay. This reference standard solution will retain its potency for at least a year and probably longer, when stored under refrigeration.

Procedure of assay - For purposes of bioassay, healthy mice weighing between 19 and 21 grams were selected from an in-bred stock strain. One ml. of the reference standard solution was placed in a 100 ml. volumetric flask and diluted to 100 ml. with water. If kept refrigerated, this stock solution containing 1 microgram per ml. will keep for several months. Aliquots of this stock solution were diluted (Columns 1 and 2, Table 1) with distilled water until the median death time of a few mice, injected intraperitoneally with 1 ml., fell between five and seven minutes. Care was taken to maintain the acidity of the dilutions at pH 4.5 or below, preferably between pH 2 and 4. Ten mice were injected with each of the dilutions which gave death times between 5 and 7 minutes in the preliminary tests. Actually, three different dilutions were used and the assay was repeated one or two days later on the same dilutions in order to provide data on the reproducibility of the assay. The entire procedure was also repeated several days later, starting with another aliquot of the reference standard solution. The median death time was then determined for each group of ten mice used on each of the dilutions. From the median death time for each dilution, the number of mouse units contained in one ml. was determined from Sommer's Table ^a. A curve relating death time to mouse units may be constructed as follows from Sommer's data. Death times of 4, 5, 6, 7, and 8 minutes are equivalent to 2.5, 1.92, 1.5, 1.39 and 1.25 mouse units respectively. If the logarithm of the dose is plotted against the reciprocal of the time, a straight line is obtained and the dose can be calculated directly from the following equation: (7)

$$\text{Log dose} = \left(\frac{1.45}{t} \right) - 0.2$$

Where t = time of death in seconds. The equation is very good for death times between 240 and 480 seconds (4 and 8 minutes).

^a Furnished by Sommer to other workers in this field. These tables are based on graphs recorded in the literature (8), and have been included in the interim plan (13) issued by the Public Health Service. A mouse unit is defined as the amount of poison that will kill a 20 gram mouse in 15 minutes.

Determination of CF value - By dividing the micrograms of poison in one ml. of dilution by the mouse units per ml. as determined from Sommer's Table a factor was obtained. The factors obtained for each dilution were averaged to arrive at a value (hereafter termed the conversion factor or the CF value), expressing the micrograms of poison equivalent to one mouse unit. In calculating this CF value, all factors obtained on any one dilution were used whenever one or more groups of ten mice had a median death time between 5 and 7 minutes.^b On the other hand, if a group of ten mice were injected with a dilution which in all trials gave a median death time less than 5 or greater than 7 minutes, these factors were not used in calculating the CF value. This conversion factor was subsequently used in calculating the micrograms of poison present in clam extracts. Typical data for determination of the CF value are summarized in Table 1.

Assay of clams - In bioassaying clams, the method of preparing and extracting the clam meats was essentially the same as that used by Hedcof (3), et al., except that, as in the use of the reference standard solution, dilutions of the acid clam extract were made so as to obtain death times between 5 and 7 minutes. In brief, the clams were shucked, washed in fresh water, drained on a sieve for 5 minutes, and thoroughly minced in a blender or a meat grinder. A 100 gram portion of the minced meat was suspended in 100 ml. of 0.1 N hydrochloric acid and boiled gently for 5 minutes with continuous stirring. The mixture was then cooled, adjusted to a pH between 4.0 and 4.5 by addition of a few drops of 5 N hydrochloric acid or 0.1 N sodium hydroxide, and made up to 200 ml. with distilled water. The supernatant liquid was clarified by settling or centrifuging, and used for injection. When large dilutions of the acid extract were required because of high toxicity, the pH of the dilutions were checked and acidified, if necessary, by the dropwise addition of dilute hydrochloric acid (0.1 or 0.01 N) to a pH between 3.5 and 4.5 as determined by congo red test paper. A few test mice were inoculated intraperitoneally to determine the dilution required to give death times between five and seven minutes. Then three mice were similarly inoculated with this dilution and the median death time was determined. From the median death time of the three mice, the corresponding number of mouse units was determined from Sommer's tables. Multiplying the number of mouse units by the dilution factor

^b

The median rather than the average death time is used because in a lot of ten mice, one or more may not die, making it impractical to calculate the average without excluding those mice that do not die. The median death time, of course, includes the use of all ten mice as long as enough die to obtain the median. Results indicate that the medians and averages do not differ significantly.

gave the number of mouse units per ml. of acid extract. The toxicity of 100 grams of meat was then determined from the number of mouse units in one ml. of extract multiplied by 200, which was the total volume of meat plus 100 ml. of dilute acid. Mouse units per 100 grams of meat were converted to micrograms of poison per 100 grams by multiplying the former by the CF value.

Each laboratory may simplify these calculations by constructing a table relating micrograms of poison per ml. of solution injected to median death times of mice. For example, the relationships shown in Table 2 hold for a CF value of 0.100 micrograms per mouse unit. The micrograms of poison in one milliliter, as read from this table, multiplied by the dilution and the total volume of the extract (200 ml.), will give directly the micrograms of poison per 100 grams of clam meat.

RESULTS AND DISCUSSION

The only method in current use for the detection and measurement of paralytic shellfish poison involves the death response of mice. Usually in the determination of quantal (all-or-none) response, an accurate determination is made of the median-effective dose (ED_{50} or LD_{50}). The LD_{50} is conveniently calculated by a moving average method (11) involving at least four consecutive dosage levels which usually differ by a constant ratio. At least four or five animals must be used for each dosage level. Thus, at least sixteen to twenty animals are required for any given assay. In actual practice, however, the concentration of the poison in shellfish has usually been measured with the aid of Sommer's table by relating the average or median death time of a few (usually 3 to 6) mice to the mouse units per volume injected.

Median death time versus LD_{50} - The comparable results obtainable by the determination of the median death time (MDT) and the LD_{50} are illustrated in Table 3 by the data obtained from two bioassays of a partially purified sample of the paralytic shellfish poison. In one assay an aliquot of one milliliter was injected intraperitoneally into mice, and in the other, two milliliter aliquots were used. Each LD_{50} determination was calculated from five different dosage levels with a ratio of 1.18-1.22 between levels. Ten animals (five male and five females) were used for each dosage level. Whenever more than half of the animals in a group died, the median death time was used to calculate the mouse units according to Sommer's table.

When the results of both methods of assay were expressed in mouse units per ml., the difference between the arithmetic averages, $15,115 \pm 2237$ (or 14.8%) "MDT" mouse units, and $16,100 \pm 1270$ (or 7.9%) LD_{50} mouse units, was not statistically significant. Likewise, when males and females were evaluated separately no significant differences in the results could be noted. It will be observed, however, that there was more variation in the assay using the median death time than in the

LD₅₀ determination. As indicated below, the precision of the median-death-time method of assay can be improved if only those dilutions are used which give median death times between 5 and 7 minutes. On this basis either bioassay technique may be used, and the sex of the mice or the volume of solution used in the injections is not critical. For routine assays a constant volume of one ml., mice of either sex, and a median death time determination have been used, since the calculations are simpler and fewer mice are required.

By definition, one mouse unit is that amount of shellfish poison which, when injected intraperitoneally into a 20-gram mouse causes death in 15 minutes. However, in actual practice with purified poison, greater precision is attained if concentrations of 1.4 to 1.9 mouse units per ml. are used, which give median death times of 5-7 minutes. With death times shorter than 5 minutes, a relatively large change in dose may produce only a small change in death time. For example, Sommer's table indicates that increasing the dose from 2.32 mouse units to 3.31 mouse units shortens the death time by only one minute. On the other hand, at death times longer than 7 minutes, rather large variations in death time may result even though the change in dose may be insignificant. As indicated in Table 1, a CF value of 0.183 micrograms per mouse unit was obtained when only median death times between 5 and 7 minutes were used. In comparison, when more dilute solutions of the same reference standard were assayed, as shown in Table 4, there was more variation in the median death times and the average CF value was significantly larger. With the larger dilutions the precision was only $\pm 20\%$ as compared with $\pm 12\%$ for the less dilute solutions in Table 1. In using dilutions giving median death times longer than 7 minutes, difficulty is also encountered if five or more mice live when a group of ten mice are injected, since it is then impossible to obtain a median death time.

A median death time is preferable to an average death time, because one or more animals may survive even when injected with solutions containing 1.4 to 1.9 mouse units per ml., and it is then impossible to calculate an average death time.

Weight of mice - It is recognized that the death time of mice injected with poison is related to the weight of the animals. Sommer and co-workers determined correction factors for use with animals of various weights above and below 20 gm., but a plateau in their weight-death time curve can be noted at and above about 23 grams. The factors range from 0.50 for a 10 gm. mouse to 1.12 for a 30 gm. mouse. In the determination of the CF value, it is suggested that 19-21 gram mice be used with no correction for the weight of the animals, since the correction factor is only 0.97-1.03, and the resulting error is less than $\pm 3\%$. Some tests were done with mice weighing 25-30 grams using Sommer's corrections for the size of the mice. All of these animals survived when either the 1:330 or 1:350 dilutions of the

reference standard solutions were used. For a CF value of 0.183 micrograms per mouse unit, the 1:330 dilution containing 0.303 micrograms per ml. should have given a median death time of approximately 6 to 7 minutes, which would correspond to 1.5 to 1.7 mouse units when corrected for 25 to 30 gram animals. On this basis it would appear that animals larger than 25 grams should not be used in the bioassay for paralytic shellfish poison.

pH - It has long been known that only acidic clam extracts can be used if the bioassay is to be meaningful. To study the effect of pH on the bioassay of the reference standard solution, two different dilutions were used. When a 1:360 dilution was injected at a pH below 4.5 all of the mice died between 5 and 8 minutes, while all of the ten mice injected with the same dilution at pH 5.5 lived. Another dilution (1:350) of the reference standard solution was also tried at a pH of 2.0 and 3.5. The median death time of ten mice on each of these solutions were respectively, 5 min. 40 sec. and 5 min. 14 sec., as compared to a median death time of 5 min. 27 sec. on the same solution at a pH of 4.7. These results indicate that the bioassay should not be done on a solution if the pH is greater than 5 and that there is no significant difference in the bioassay of solution varying in pH from 2.0 to 4.5.

CF values - A summary of the data obtained in the determination of the CF value by seven different laboratories is given in Table 5. All of the laboratories collaborating in this study used the bioassay procedure described herein, and the mean CF values were calculated from the factors selected according to the previously outlined procedure. The mean CF values obtained averaged 0.191 and ranged from 0.164 to 0.250.

To determine if the range of mean values is unusually large in comparison to the variation of replicate values within a given laboratory, analysis of variance techniques were employed. Since the standard deviation of laboratory F is small in comparison to the other laboratories, it was excluded and only laboratories A, B, C, D, E, and G were tested for homogeneity of means. To do this the variation between laboratory means was compared with the variation of values within given laboratories. The ratio of these two variances was 13.5. Since this value is significantly greater than one, it can be concluded that there are certain real differences between the mean CF values obtained by this group of collaborating laboratories. Subsequent comparisons brought forth that the mean CF value determined by laboratory A is significantly higher than any of the other mean values. The mean CF values from laboratories E and F do not differ significantly from each other and occupy a median position, followed by laboratories B, D, C, and G, which were a homogeneous group with the lowest average CF values. The standard deviations of all the laboratories were roughly comparable, except for laboratory F which had significantly better precision than the others.

These results verify the need for standardizing the bioassay by means of a purified reference standard for intra-laboratory checking, and indicate that significant differences may be obtained which are probably dependent upon environmental factors, the strain of mice used, or inadvertent deviations from the prescribed technique.

If each laboratory were to assay the same batch of clams, and if mouse units were converted to micrograms of poison by using the CF value obtained in each laboratory, it is assumed that results not differing significantly from each other would be obtained. This deduction needs to be confirmed, however, by a collaborative study similar to the above.

Shellfish assay - Initially, in bioassaying clam extracts, ten mice were injected with the dilution giving a median death time between 5 and 7 minutes. As indicated in Table 6, similar results were obtained if the median death time of only the first three mice injected were used. In order to conserve mice, time, and labor, the use of three mice has been adopted for routine assays. That is to say, the injection of three mice with the dilution giving a median death time between 5 and 7 minutes is a sufficient number upon which to base the calculation of the toxicity of clams with a standard error no greater than $\pm 20\%$ of the mean. Actually, it may be necessary to use an equal or greater number of mice in preliminary tests to locate by trial and error the correct dilution.

Recovery of purified poison added to clam meat. In an attempt to estimate the accuracy of the assay it seemed desirable to obtain information on the ability of the acid extraction procedure and the bioassay to measure the actual total toxicity of clam meat. To do this, varying amounts of purified reference solution were added to samples of minced clams (nonpoisonous commercially canned clams and raw nonpoisonous clams from Yaquina Bay, Oregon) before acid extraction. The results shown in Figure 1 indicate that as the amount of poison added per 100 grams of meat is diminished toward zero the per cent that can be recovered approaches about 40 per cent. As the toxicity increases the assay becomes progressively better until the recovery approaches 100 per cent at higher toxicities where large dilutions are used for bioassay.

In another experiment, known amounts of purified poison were added to the acid extract of nontoxic razor clams from Ketchikan, Alaska. Results similar to the above were obtained. For example, after adding 0.5 micrograms of poison per ml. of acid extract, only 0.29 micrograms per ml. or 59 per cent could be recovered when assays giving death times between 5 and 7 minutes were used. If median death times longer than 7 minutes could be accepted, the recovery would appear somewhat better and might be as high as 95 per cent. However, in the determination of the CF value only the results of assays giving median death times between 5 and 7 minutes are acceptable, and therefore, assays of clams must be performed in the same way in order to determine accurately the micrograms of poison present.

The lack of sensitivity, when undiluted or slightly diluted acid extracts of low toxicity clams are injected into mice, is the most unsatisfactory feature of the bioassay.

Salt effect - In an effort to determine the reason for the poor recovery of poison in the bioassay of low toxicity clams, the effect of salt and buffers on the bioassay were studied. When a dilution of purified poison was made with 0.01 M sodium acetate (0.08%) at pH 4.0 the bioassay was not significantly different from the bioassay of the same dilution of the poison in distilled water (pH 4). On the other hand, as can be observed in Figure 2, the addition of sodium chloride to a dilution of the reference standard causes a significant lowering of the bioassay. In the presence of 0.5 per cent sodium chloride the recovery is about 69 per cent and with 1.0 per cent sodium chloride the recovery is about 55 per cent.

A similar lowering of the bioassay was observed when the ash from an acid extract of clams was added to a dilution of the purified poison. The ash or salt content of a standard acid extract of Mya arenaria clams was determined by adding three 10 ml. aliquots of the extract to each of three fired and weighed crucibles. The liquid added to the crucibles was evaporated to dryness on a hot plate and left overnight in an oven at 110°C. The dried material was then ashed in a muffle furnace at 500°C., cooled and the ash dissolved in 1 ml. of concentrated nitric acid, taken to dryness and again ashed at 500°C. The ash was then converted to the chloride salt by re-dissolving it in 1 ml. of concentrated hydrochloric acid and drying to constant weight. The salt thus obtained was dissolved in 10 ml. of a dilution of the reference standard solution which in preliminary trials gave death times falling within 5 to 7 minutes. The average ash content was 0.56 per cent and this quantity of ash from clams lowered the recovery of poison to 63.5 per cent, which is practically equivalent to the effect that would be observed if an equal quantity of sodium chloride (Figure 2) were added to the reference standard solution.

These experiments indicate that, with low toxicity acid extracts from shellfish, the insensitivity of the bioassay is at least partially due to the salt concentration of the acid extract. This difficulty is inherent in the bioassay of crude preparations that contain little poison and cannot be diluted sufficiently to nullify the salt effect and can only be solved by separating the poison from the salts through the use of cation exchange resins. This separation is the first step in the isolation of the poison and will be described in a subsequent paper on the chemical determination of paralytic shellfish poison.

SUMMARY

A procedure utilizing a purified reference standard has been developed for the bioassay of paralytic shellfish poison. The conversion factors (CF) representing the micrograms of poison per mouse unit reported by seven different laboratories using the purified poison and the procedures outlined in this paper, range from 0.164 to 0.250. These values can be divided into three groups having CF values of 0.250, 0.195 to 0.206, and 0.164 to 0.183, and it is shown that these groups differ significantly from each other. These results verify the need for standardizing the bioassay by means of a purified reference standard and indicate that significant differences may exist due to the strain of mice or uncontrolled environmental conditions.

In the determination of the CF value, as well as in the bioassay of an acid extract of clams, it has been demonstrated that: the relationship between mouse units and median death time can be used equally as well as an LD₅₀ value; the pH of the solution injected into the mice should be between 2 and 4; dilutions should be made so that the median death time falls between 5 and 7 minutes; the weight of the mice used should be preferably between 19 and 21 grams, and not over 23 grams; a total of ten mice should be used with each dilution in the determination of the CF value; and for the routine assays the injection of three mice is sufficient provided the median death time falls between five and seven minutes.

Recovery studies indicate that in assaying low toxicity clams (around 400 mouse units per 100 grams) the bioassay may underestimate the toxicity by as much as 60 per cent. A 60 to 70 per cent reduction of the bioassay was observed also when the ash from an equivalent amount of an acid extract of clams or 0.5 per cent sodium chloride was added to a solution of purified poison. Salt accounts, at least in part, for the poor recovery in the bioassay of low toxicity clams.

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REFERENCES

1. Bennett, B. M., Estimation of LD₅₀ by Moving Averages, J. Hyg. 50, No. 2, 157 (1952).
2. Magnusson, H. W., and Carlson, C. J., Technological Studies on the Alaska Butter Clam; Review of the Problems of Occurrence of a Toxin, Fisheries Experimental Commission of Alaska; Technical Report No. 2 (1951).
3. Hedcof, J. C., Paralytic Shellfish Poisoning on the Canadian Atlantic Coast, Bull. Fisheries Research Board of Canada. 75, (1947).
4. Meyer, K. F., Food Poisoning, New England Journal of Medicine. 249, 848 (1953).
5. Meyer, K. F., Sommer, H., and Schoenholz, P., Mussel Poisoning, J. Preventive Medicine. 2, 365 (1928).
6. Mold, J. D., Schantz, E. J., et al, Evidence for the Purity of the Poison Isolated From Toxic Clams and Mussels. J. Am. Chem. Soc., 79 5235 (1957)
7. Schantz, E. J., et al, A Procedure for the Isolation and Purification of the Poison From Toxic Clams and Mussel Tissue, J. Am. Chem. Soc., 79 5230 (1957).
8. Sommer, H., Meyer, K. F., Paralytic Shell-Fish Poisoning, Arch. Path. 24, 560 (1937).
9. Sommer, H., Whedon, W. F., Kofoid, C. A., and Stohler, R., Relations of Paralytic Shellfish Poison to Certain Plankton Organisms of the Genus Gonyaulax. Arch. Path. 24, 537 (1937).
10. Tennant, A. C., Naubert, J., and Corbell, H. E., An Outbreak of Paralytic Shellfish Poisoning, Canadian Medical Assoc. J. 72, 436 (1955).
11. U. S. Dept. of Health, Education, and Welfare - Tentative Standard Procedure for the Determination of Shellfish (Mussels or Soft Clams) Poison, Adopted November 19, 1943. Mimeographed procedure. Four pages, with suggestions of 1950, Public Health Service, Washington 25, D. C.
12. U. S. Dept. of Health, Education, and Welfare - Conference on Shellfish Toxicology. Mimeographed pamphlet, 35 pages, May 25, 1955, Public Health Service, Washington 25, D. C.
13. U. S. Dept. of Health, Education, and Welfare - Interim Plan for Standardizing the Bioassay of Paralytic Shellfish Poison by Use of A Reference Standard, May 7, 1956. Public Health Service, Washington 25, D.C.

Table 1.-- Determination of factor for conversion of mouse units to micrograms of poison

ml. stock solution (1 ug/ml.)	ml. of water	Dilution of reference std.	ug. per ml.	Median death time	Mouse units per ml. (from Sommer's table)	ug. per mouse unit ¹
10	24	1:340	.294	5:23	1.78	0.165
10	24	1:340	.294	6:24	1.53	0.192
10	25	1:350	.286	6:37	1.46	0.196
10	25	1:350	.286	5:16	1.83	0.156
10	25	1:350	.286	6:50	1.42	0.201
10	25	1:350	.286	5:27	1.74	0.164
10	25	1:350	.286	6:03	1.60	0.179
10	26	1:360	.278	5:52	1.64	0.170
10	26	1:360	.278	8:07	1.24	0.224

¹ CF value = 0.183 ug. per mouse unit $\pm$.022 (or 12%)

Standard error of mean = $\frac{.022}{\sqrt{9}} = .007$

Table 2. -- Relationship of death time to micrograms of shellfish poison for a CP value of 0.13

Median death time Min. Seconds	ug. Poison per ml.	
	Min.	Seconds
0.13	22.1	00
0.13	5	05
0.13	5	10
0.13	5	15
0.13	5	20
0.13	5	30
0.13	5	40
0.13	5	50
0.13	6	00
0.13	6	15
0.13	6	30
0.13	6	45
0.13	7	00
0.13	22.1	00

Table 3. -- Comparison of MDT and LD₅₀ methods for the bioassay of paralytic shellfish poison

ml. Injected	Dilution ¹	Average weight	MDT Min. Sec.	Sommer's mouse units per ml.	No Mice dead Injected	LD ₅₀
1	1:11,000	20.6	7:40	14,300	7/10	15,200
1	1:13,000	21.4	22:35	12,285	6/10	
1	1:15,000	19.7			5/10	
1	1:17,000	21.2			5/10	
1	1:19,000	20.9			0/10	17,000
2	1:22,000	23	6:07	17,270	8/10	
2	1:26,000	21	10:28	14,170	7/10	
2	1:30,000	22	8:55	17,550	9/10	
2	1:34,000	21			5/10	
2	1:38,000	20			2/10	

¹ Partially purified shellfish poison solution

Table 4. -- Effect of injecting dilutions giving median death times longer than seven minutes

Dilution ¹	ug. per ml.	Median death time	House units per ml.	ug. per house unit ²
1:380	.263	17:37	0.976	.269
1:360	.263	5:51	1.64	.160
1:400	.250	9:49	1.18	.212
1:400	.250	19:20	0.963	.259
1:400	.250	6:32	1.48	.169
1:420	.238	10:05	1.11	.214
1:420	.238	8:02	1.25	.190

¹Ten mice injected with each dilution.

²CF value .21 ± .0418 (or 20%) based on average of 7 values in this column.

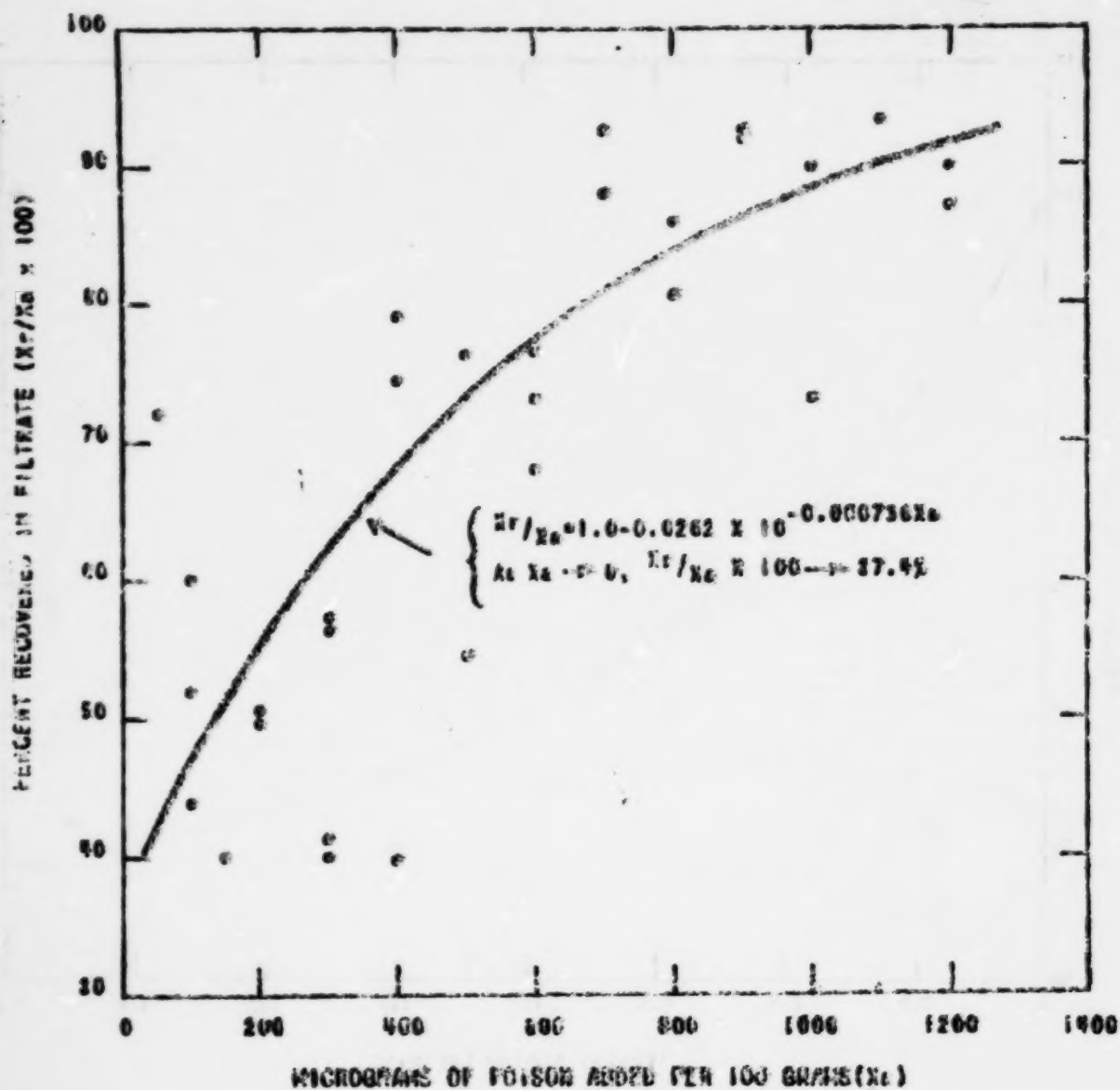
$$\text{Standard error of mean} = \frac{.0418}{\sqrt{7}} = .016$$

Table 5. -- Comparison of CF values obtained by several collaborating laboratories¹ assaying the reference standard solution

Laboratory	A	B	C	D	E	F	G
Factors	.189	.231	.165	.232	.244	.208	.194
	.250	.201	.192	.191	.224	.200	.187
	.202	.188	.196	.173	.231	.184	.176
	.258	.153	.156	.169	.192	.201	.179
	.284	.174	.201	.178	.176	.209	.168
	.244	.177	.164	.140	.245	.206	.178
	.263	.150	.179	.165	.248	.197	.148
	.163	.160	.170	.154	.164	.188	.205
	.255	.164	.224	.153	.160	.188	.150
	.196	.154		.148	.178	.173	.150
	.300	.136		.152			.144
	.310	.206		.140			.182
				.165			
				.130			
(CF value Mean of Factors	.250	.175	.133	.164	.206	.195	.172
Std. dev.	±.046	±.028	±.022	±.025	±.036	±.012	±.020
% Error (Std. dev./Mean x 100)	18	16	12	15	17	6.2	12

¹Collaborating Laboratories: Food and Drug Laboratory, Depart. of National Health & Welfare, Vancouver, B.C.; Laboratory of Hygiene, Dept. of National Health & Welfare, Ottawa, Canada; Chemical Corps, Chemistry Branch, Fort Detrick, Frederick, Md.; Food & Drug Administration, Division of Pharmacology, Washington 25, D.C.; Arctic Health Research Center, Public Health Service, Anchorage, Alaska; Food and Drug Laboratory, Dept. of National Health & Welfare, Tunney's Pasture, Ottawa, Ontario; and Robert A. Taft Sanitary Engineering Center, Public Health Service, Cincinnati 26, Ohio.

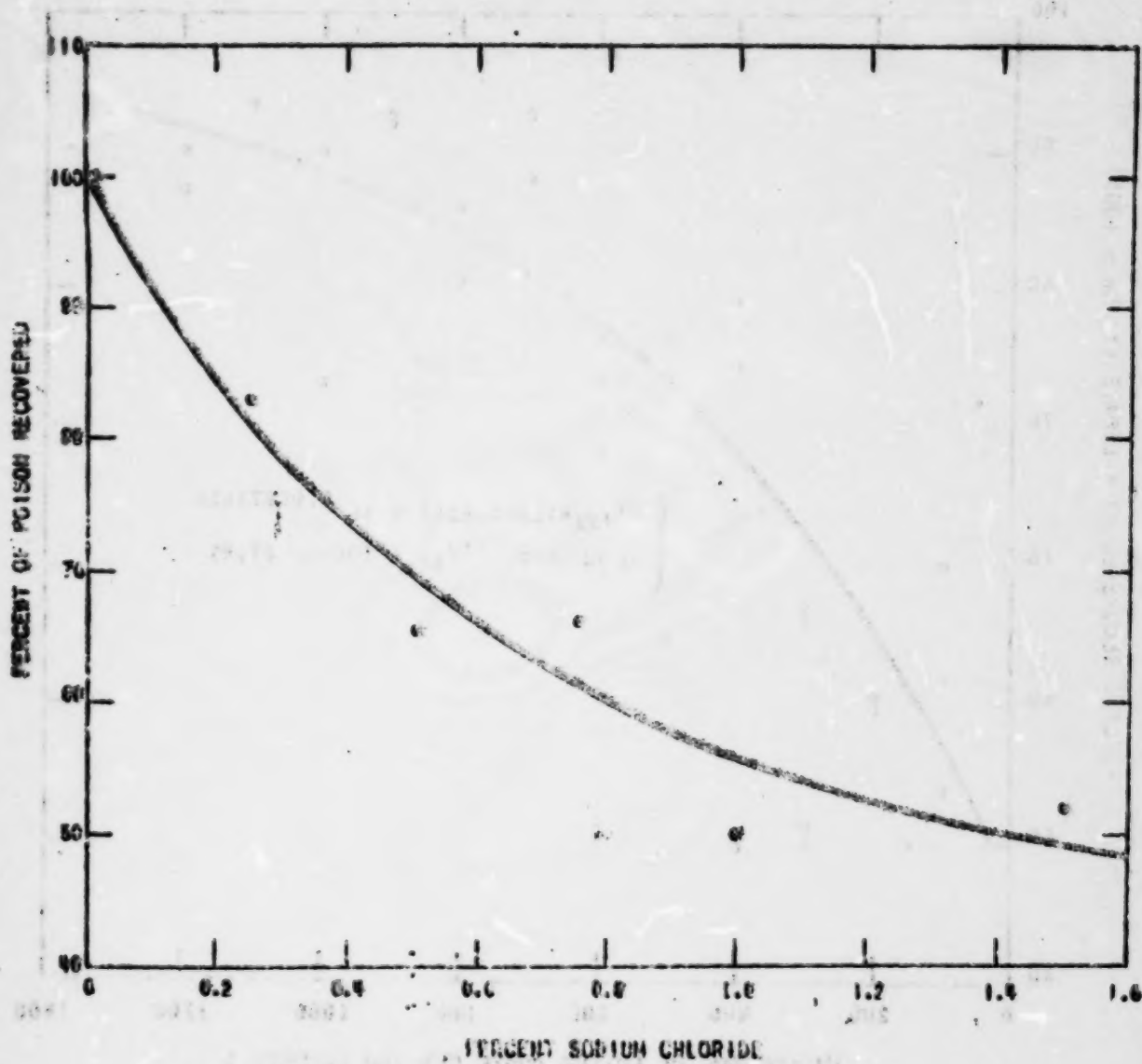
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RECOVERY OF SHELLFISH POISON ADDED TO NON-POISONOUS CLAMS

Figure 1

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EFFECT OF SALT ON THE BIOASSAY OF SHELLFISH POISON

Figure 2

A P P E N D I X B

POISONOUS FISH-LIKE VERTEBRATES

By

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The military significance of poisonous fishes has long been recognized by physicians concerned with naval medical problems. Alexander the Great forbade his soldiers to eat fish during conquests because he believed that some fishes produced skin disease. Fonssagrives and de Mericourt (1861) and Fonssagrives (1877) were the first to prepare an actual military manual on poisonous fishes. Their work was done under sponsorship of the French Navy. Included in this manual is the report of a single outbreak involving 50 crew members, the result of eating a single sardine dinner. Recognition of the problem of fish poisoning and its deleterious effects upon the health of the Russian Imperial Navy was indicated by the writing of Savitschenko's (1886) great treatise on poisonous fishes. Hiyama (1943) conducted his survey on poisonous fishes in the Marshall and Mariana Islands under the auspices of the Japanese Imperial Navy. It has been estimated that during World War II more than 400 Japanese military personnel succumbed to fish poisoning in Micronesia. Hiyama's work resulted in a series of profusely illustrated survival handbooks which could be used to identify poisonous fishes. Spear (1904), Kerr (1912), Walker (1922), Gregory (1925), Neilson (1927), O'Neill (1938), Mann (1933), Gilman (1942), Vonfraenkel and Krick (1945), Schneck (1945), Cohen, Emert and Goss (1946), Halstead (1951a,b), Halstead and Lively (1954), and Fish and Cobb (1954) have discussed fish poisoning and their significance to American military personnel. In addition to the previously mentioned publications, numerous field bulletins and medical manuals have considered the subject of poisonous fishes to a lesser or greater extent. Unfortunately, until recent years little research has been conducted on the subject; consequently the problem has been difficult to define.

TERMINOLOGY

The nomenclature dealing with poisonous fish-like vertebrates and the intoxications which they produce has been the subject of much confusion. Ichthyotoxism is the general term which should be used to include the forms of intoxication resulting from contact with both poisonous and venomous fish-like vertebrates. Ichthyotoxic fish-like vertebrates may be classified into two major groups: poisonous fish-like vertebrates, which produce ichthyosarcotoxism, ichthootoxism, or ichthyohaemotoxism, and venomous fish-like vertebrates, which produce ichthyoacanthotoxism. Venomous fish-like vertebrates are discussed in a separate medical manual.

Poisonous Fish-like Vertebrates. Fish-like vertebrates which when ingested by humans produce various gastro-intestinal and/or neurological disturbances due to a toxic substance present within their body are termed poisonous. This system of classification is not concerned with bacterial food poisonings resulting from the ingestion of "contaminated" fish-like vertebrates. The only known modification to the foregoing statement is scombroid poisoning in which bacteria are believed to be a factor in the production of the toxic agent. Since the chemical characteristics of fish poisons have not been defined, poisonous fish-like vertebrates have been tentatively classified on the basis of their phylogenetic relationships and symptomatology of the intoxication which they produce. It will be noted that certain fish groups are classified under more than one category.

The weaknesses of this somewhat artificial system are recognized, but it appears to be the most practical classification at present. Future chemical studies will undoubtedly alter this system. Poisonous fish-like vertebrates may be subdivided into the following clinical categories:

A. Ichthyosarcotoxism: The specific term used to designate an intoxication resulting from the ingestion of the flesh of poisonous fish-like vertebrates. The origin of ichthyosarcotoxins, or the exact mechanism by which fishes become poisonous is unknown. The poison may have a wide distribution within the body of the fish, i.e., musculature, liver, gonads, intestines, intestinal contents, blood, etc., and any one or a combination of these parts may cause intoxication. The poison(s) involved would be termed ichthyosarcotoxin(s). The following groups of fish-like vertebrates are believed to contain ichthyosarcotoxic members:

1. Cyclostomata: The slime and flesh of certain lampreys and hagfishes are reported to produce a gastro-intestinal upset. The clinical characteristics of the intoxication produced by this group have not been completely defined. The disease is termed cyclostome ichthyosarcotoxism, or cyclostome poisoning.
2. Elasmobranchii: The flesh and viscera of some sharks and rays produce severe intoxications. The gastro-intestinal symptoms usually predominate in mild cases, but the neurological symptoms are most pronounced in severe intoxications. Death is believed to be by respiratory paralysis. The disease is termed elasmobranch ichthyosarcotoxism, or elasmobranch poisoning.
3. Chimaerae: The musculature and viscera of certain chimaeroids have been incriminated as containing a neurotoxin. Present clinical information is far too meager to come to any conclusions regarding the nature of the poison. The disease is termed chimaeroid ichthyosarcotoxism, or chimaeroid poisoning.
4. Apodes: The musculature and viscera of some of the muraenid eels contains a violent poison, the ingestion of which produces violent neurotoxic symptoms with motor paralysis and convulsions predominating. The case fatality rate is estimated to be about 10 per cent or more, and death results from respiratory paralysis. The disease is termed gymnothorax ichthyosarcotoxism, or gymnothorax poisoning.
5. Scombroidea: Some of the scombroids, tuna, skipjack, bonito, etc., may on rare occasions cause ciguatera, but usually when poisoning results from them it produces an entirely different form of intoxication. This is the only type of fish poisoning in which bacteria appear to play an etiological role in the formation of the toxin.

If scombroids are inadequately preserved, a toxic "histamine-like" substance is formed, possibly from the decarboxylation of histidine, a normal constituent of fish flesh. The symptoms most often present are nausea, vomiting, flushing of the face, swelling of the lips, and urticaria, which are typical of a histamine reaction. For some unknown reason scombroid fishes appear to be more conducive to producing intoxications of this type than other fishes. The disease is termed scombroid ichthyosarcotoxism, or scombroid poisoning.

6. Tetraodontoidea: The most violent form of fish poisoning is produced by tetraodontoid fishes. The disease is characterized by rapid developing and violent symptoms. Predominant symptoms consist of paresthesias, motor paralysis, convulsions, and death by respiratory paralysis. The case fatality rate is about 61 percent. The disease is termed tetraodon ichthyosarcotoxism, or tetraodon poisoning.

7. Ciguatera-producing Teleostei: More than 325 species of marine fishes which are widely distributed throughout the Orders Isospondyli, Apodes, Mesichthyes, and Acanthopterygii. Fishes included within this category contain a poison in their flesh and viscera which when ingested causes gastro-intestinal and neurological disturbances. Paresthesias, extreme weakness, joint aches, myalgia and paradoxical sensory disturbances predominate the clinical picture. The case fatality rate is about 7 per cent, and death is by respiratory paralysis. The disease is termed the ciguatera type of ichthyosarcotoxism, or merely ciguatera.

- *. Gempylidae: Some of the gempylids or escolarers contain an oil within their flesh and bone marrow which has a pronounced purgative effect when ingested. Diarrhea develops rapidly, and is said to be without cramping or pain. Since there are no other untoward effects ingestion of the oil can hardly be considered as an intoxication in the usual sense of the word. It may be termed gempylid "poisoning", but preferably gempylid diarrhea.

B. Ichthyootoxism: The specific term used to designate intoxications resulting from the roe of fishes. The poison is, for the most part, restricted to the gonads, and is usually found in no other part of the fish. The roe of numerous species of fresh water and some marine fishes contains a toxic substance during the reproductive period. There appears to be a definite relationship between gonadal activity and toxin production. This relationship is also observed in poisonous tetraodontoid fishes and other ichthyosarcotoxic fishes, but in these instances the toxin is widely distributed throughout the body of the fish, which is not the case in ichthyootoxic fishes. The poison(s) involved would be termed ichthyootoxin(s). The following groups are believed to contain ichthyootoxic members.

1. Acipenseroidel, Subholostei, Ostariophys contain the bulk of the freshwater fishes which have been incriminated to date. The most common offenders are the cyprinoid or minnow-like fishes. Ingestion of the roe may result in violent gastro-intestinal reactions, convulsions and death. The disease is best designated by the generic name of the fish involved, i.e., Schizothorax ichthyootoxism, Barbus ichthyootoxism, etc., or merely fish roe poisoning.

2. Cottidae: A single marine cottoid, Scorpaenichthys marmoratus, species has been incriminated as an ichthyootoxic fish. The symptomatology is similar to that produced by freshwater species. The disease is termed Scorpaenichthys ichthyootoxism, or merely fish roe poisoning.

C. Ichthyohaemotoxism: The specific term used to designate an intoxication resulting from the ingestion or injection of fresh fish blood. Cooking and contact with gastric juices destroy the poison in most instances. The principal general symptoms are those of a gastro-enteritis accompanied by paresthesias, paralysis, and sometimes death. Raw eel blood placed on the tongue or in the eye is said to result in a severe inflammatory response. Ichthyohaemotoxism is exceedingly rare, and among the least known of zoolntoxications. The poison(s) involved would be termed ichthyohaemotoxin(s). The following groups of fish-like vertebrates are believed to contain Ichthyohaemotoxic members:

1. Apodes: The bulk of the research that has been conducted to date has incriminated members of the eel families, Anguillidae, Congridae, Muraenidae, and Ophichthyidae. The disease is designated by the generic name of the fish species involved, i.e., Muraenophis ichthyohaemotoxism, Anguilla ichthyohaemotoxism, etc., or merely fish blood poisoning.
2. Miscellaneous groups: Petromyzonidae, Torpedinidae, Xiphiidae, Thunnidae, and Cyprinidae. Toxicological data is too meager to be certain of their exact clinical classification. However, the blood of some of the members of these families is believed to be capable of producing ichthyohaemotoxism. The method of designating the disease is similar to that used in the preceding group.

CYCLOSTOMES

Class AGNATHA: Jawless Fish-like Vertebrates

Subclass CYCLOSTOMATA: Hagfish and Lampreys

Cyclostomes are a group of fish-like vertebrates, having an eel-like form, cartilaginous or fibrous skeletons, no definite jaws or bony teeth, and a cranium of a primitive type. There are no pelvic girdles, paired limbs, nor true ribs. There are 6 to 14 pairs of gill pouches opening either directly into the pharynx or into a separate respiratory tube. Only a single nostril is present. The skin is scaleless. Because of their structural simplicity, cyclostomes are generally considered the most primitive of true vertebrates.

Species:

Myxine glutinosa Linnaeus (Fig.) - Borer, hagfish, poison ramper. North Atlantic.

Caspionyzon wagneri (Kessler) (Fig.) - Caspian nine-eye. Caspian Sea, rivers of the Caspian basin.

Lampetra fluviatilis (Linnaeus) (Fig.) - River lamprey. Europe, Russia.

Lampetra planeri (Bloch) (Fig.) - River Lamprey. Basin of the Baltic Sea, Dnieper, and Don Rivers

Petromyzon marinus Linnaeus (Fig.) - Sea Lamprey. Coasts and rivers of both sides of the Atlantic, rivers of Mediterranean Sea.

Toxicology

The slime and flesh of the preceding species are reputed to contain substances, resulting in a gastro-intestinal upset. There is no other data available (Engelsen 1922).

ELASMOBRANCHS

Class CHONDRICHTHYES: Elasmobranchs and Chimaeroids

Subclass ELASMOBRANCHII: Sharks, Skates, and Rays

Order SELACHII: Sharks

The order Selachii, which includes modern sharks, consists of a group of fish-like vertebrates which have gill openings that are at least partly lateral, and the edges of the pectoral fins are not attached to the sides of the head anterior to gill openings. The upper edges of the orbits are free from the eyeballs as free eyelids.

Species:

Carcharinus melanopterus (Quoy and Gaimard) (Fig.) - Black-tipped sand shark. Indo-Pacific, South Africa to the East Indies, Hawaiian, Tuamotu, and Marianas Islands.

Galeorhinus galeus (Linnaeus) (Fig.) - Dogfish. Temperate and tropical seas, eastern Atlantic north to the British Isles, and Scandinavia, and south to the Cape of Good Hope.

Prionace glauca (Linnaeus) (Fig.) - Blue shark. Cosmopolitan, in tropical, subtropical, and warm-temperate belts of all oceans.

Somniosus microcephalus (Bloch and Schneider) (Fig.) - Arctic Atlantic, North Sea east to the White Sea and west to the Gulf of St. Lawrence, Greenland.

Heptranchias perlo (Bonnaterre) (Fig.) - Seven-gilled shark. East and west Atlantic, Mediterranean, Cape of Good Hope, Japan.

Hexanchus griseus (Bonnaterre) (Fig.) - Six-gilled shark, cow shark, grey shark, or mud shark. Atlantic, Pacific coast of North America, Chile, Japan, Australia, Southern Indian Ocean and South Africa.

Carcharodon carcharias (Linnaeus) (Fig.) - White shark, Man-eater. Cosmopolitan, in tropical, subtropical and warm-temperate belts of all oceans.

Cephaloscyllium uter (Jordan and Gilbert) (Fig.) - Small shark. Monterey Bay to Lower California.

Scyliorhinus caniculus Linnaeus (Fig.) - Lesser-spotted Dogfish. Atlantic Ocean northward from West Africa to the British Isles and Scandinavia, Mediterranean Sea.

Scyliorhinus stellaris Linnaeus (Fig.) - Nursehound shark. Eastern Atlantic and Mediterranean Sea.

Sphyrna tiburo (Linnaeus) (Fig.) - Hammerhead shark. Tropical to warm-temperate belt of the Atlantic from southern Brazil to North Carolina, West Africa; west coast of America, southern California to Ecuador.

Sphyrna zygaena (Linnaeus) (Fig.) - Hammerhead shark.
Tropical to warm-temperate belt of the Atlantic and Pacific Oceans.

Squatina dumeril (Lesueur) (Fig.) - Monk-fish, angel shark
East coast of the United States, Gulf of Mexico.

Mustelus canis (Mitchill) (Fig.) - Smooth Dogfish
Western Atlantic from Cape Cod southward to the Caribbean and Uruguay.

Clinic

Clinical Characteristics: Our knowledge of the symptoms produced by elasmobranch poisoning is based almost entirely upon the observations of Sauvages (1764), Autenrieth (1833), Coutaud (1879), Jensen (1914), and Baje (1939). The most severe forms of intoxication usually result from the ingestion of the liver. The musculature is also reported as mildly toxic with the symptoms seldom more than that of a mild gastro-enteritis with a predominating diarrhea. Ingestion of toxic liver results in the development of symptoms usually within a period of 30 minutes, consisting of nausea, vomiting, diarrhea, abdominal pain, headache, rapid weak pulse, malaise, cold sweats, oral paresthesias, and burning sensation of the tongue, throat, and esophagus. As time progresses, the neurological and other symptoms become more pronounced, resulting in extreme weakness, trismus, muscular cramps, sensation of heaviness of the limbs, blepharospasm, dilatation of pupils, hiccoughs, visual disturbances, joint aches, delirium, ataxia, incontinence, dysuria, respiratory distress, coma and death. The recovery period varies from a day or two to several weeks. Records are too incomplete to determine the mortality rate. Again, the relationship, if any, of elasmobranch poisoning to the ciguatera type of ichthyosarcotoxism is not understood.

Pathology: Coutaud (1879) includes a single autopsy report of one of a series of seven victims. Aside from visceral congestion, no unusual pathological findings were noted.

Treatment: There is no known antidote. Treatment is symptomatic.

Prevention: Shark livers should not be eaten, particularly in large specimens.

TOXICOLOGY

The toxic effects of Somniosus microcephalus, the Greenland shark, have been studied largely by Baje (1939). He observed that dogs intoxicated by Somniosus walk with slow, stiff steps, develop hypersalivation, vomiting, explosive diarrhea, conjunctivitis, muscular twitching, turning of the eyes upward and outward, respiratory distress, tonic and clonic convulsions and death. The diarrhea which is said to be characteristic of the Greenland shark-meat intoxications is almost always absent in the more serious cases of intoxications produced by warm water sharks. Gradual

habituation to toxic Somnolus flesh "sometimes gives a certain degree of immunity from the toxin." Physical work is said to precipitate the appearance of the symptoms. Dogs which have eaten poisonous shark meat without ill effect become sick when they pull a sledge. Exertion results in the dog's gasping for breath, perspiration, salivation, ataxia and collapse. After resting the dogs may partially recover, stagger to their feet, and continue on for a period of time.

It is said that shark meat can be detoxified by cutting it into strips and hanging it up in the open air to dry. Detoxification is most complete when the meat is hung up during the winter, and frozen. Later when it thaws the fluid drains off, and in time the sun and wind dry it thoroughly. Meat that is incompletely dried during the summer months may form a superficial crust which prevents the inner portions from becoming dry. Poorly dried meat is said to be more toxic than fresh meat. Moreover, if dogs are fed both properly dried and fresh shark meat there appears to be a synergistic effect, resulting in a more serious intoxication than if fresh shark meat were used alone. Apparently drying does not destroy the toxin, but merely inactivates the toxin in the dried meat.

The toxicity of Somnolus seems to vary according to the locality, the time of year, and the individual specimen. Ordinary cooking procedures do not destroy the toxin, but it can be removed by boiling the meat in several changes of water.

The toxin appears to be primarily a parasympathomimetic substance. At least the physiological evidence lends credence to Baje's conjecture that the toxin may be related to the choline available in muscle as a component of lecithin. The similarity of choline to muscarine is also suggested as an explanation of the mode of action of the toxin. There is no other data available regarding shark poisons.

CHIMAERIDS

Subclass HOLOCEPHALI

Order CHIMAERAE: Chimaeroids

Chimaeroids, elephantfish, or ratfish, as they are sometimes called, are a group of cartilaginous fishes having a single external gill opening on either side covered over by an opercular skin fold with cartilaginous supports leading to a common branchial chamber into which the true gill clefts open. Externally, chimaeroids are more or less compressed laterally tapering posteriorly to a slender tail. The snout is rounded or conical, extended as a long pointed beak, or bearing a curious hoe-shaped proboscis. There are two dorsal fins. The first is triangular, usually higher than the second, and edged anteriorly by a strong sharp-pointed bony spine which serves as a venom apparatus.

Species:

Chimaera monstrosa Linnaeus (Fig.) - Elephant fishes, chimaeras. North Atlantic from Norway and Iceland to Cuba, the Azores, Morocco, and the Mediterranean Sea, also South Africa.

Hydrolagus affinis (Capello) (Fig.) - Atlantic Ocean, from Cape Cod to Portugal.

Hydrolagus coliei (Lay and Bennett) (Fig.) - Pacific Ratfish. Pacific coast of North America.

Medical Aspects

The liver and roe of chimaeras is reputed to be poisonous in some localities. Toxicological tests on the reproductive organs of Hydrolagus coliei have been found to be poisonous by mouse intraperitoneal tests. There is no reliable clinical data available. However, it is suggested that the viscera of these fishes be eliminated from the diet.

APODES

Order APODES: Eels

The order Apodes, or true eels, includes a group of fishes which have the shoulder-girdle free from the skull, and the bones of the jaws reduced in number by coalescence of the parts. Their body is anguilliform and destitute of ventral fins. Ichthyosarcotoxic eels are primarily members of the apodal family Muraenidae, the moray eels. The body of these fishes is scaleless, elongate, and rounded or more or less compressed. The dorsal and anal fins are confluent with the caudal, and are generally covered by thick skin. The pectoral and pelvic fins are absent.

Species:

Echidna nebulosa (Ahl) (Fig.) - Mottled or spotted eel. East Africa to Netherlands Indies, Australia, China, Hawaiian Islands, and Tahiti.

Gymnothorax eurostus (Abbott) (Fig.) - Morayed eels. Indo-Pacific area.

Gymnothorax flavimarginatus (Ruppell) (Fig.) - Morayed eels. Hawaiian Islands, westward to East Africa.

Gymnothorax funebris (Ranzani) (Fig.) - Morayed eels. Caribbean Sea south to Brazil.

Gymnothorax javanicus (Bleeker) (Fig.) - Moray eels.
East Africa to the Hawaiian Islands.

Gymnothorax meleagris (Shaw and Nodder) (Fig.) - Moray eels.
East Africa to the Hawaiian Islands, Japan south to
Australia.

Gymnothorax moringa (Cuvier) (Fig.) - Moray eels.
Gulf of Mexico to Brazil.

Gymnothorax petellii (Bleeker) (Fig.) - Moray eels.
Hawaiian Islands to the Red Sea.

Gymnothorax pictus (Ahl) (Fig.) - Moray eels.
Polynesia to East Africa.

Gymnothorax tile (Hamilton & Buchanan) (Fig.) - Moray eels.
Philippine Islands and Indian Ocean.

Gymnothorax undulatus (Lacepede) (Fig.) - Moray eels.
Hawaiian Island to the Red Sea, and East Africa.

Muraena insularum Jordan and Davis (Fig.) - Moray eels.
Eastern Pacific Ocean.

Muraena lentiginosa Jenyns (Fig.) - Moray eels.
Pacific coast of America, from Gulf of California to the
Galapagos Islands.

Murenophis helena (Linnaeus) (Fig.) - Moray eels.
Eastern Atlantic Ocean and Mediterranean Sea.

Medical Aspects

Clinical Characteristics: Symptoms of tingling and numbness about the lips, tongue, hands, and feet, with a feeling of heaviness in the legs, usually develop within 20 minutes to 8 hours after ingestion of the toxin. These symptoms may be followed by nausea, vomiting, diarrhea, abdominal pain, malaise, metallic taste, sore throat, laryngeal paralysis, meningismus, laryngeal spasm, aphonia, excessive mucus production, foaming at the mouth, intense perspiration, increased body temperature, conjunctivitis, paralysis of the respiratory muscles, ataxia, general motor incoordination, trismus, violent clonic and tonic convulsions, abnormal deep and superficial reflexes, coma, and death. Sensory examinations are usually normal to deep and superficial pain.

Characteristic signs of this form of intoxication appear to be the absence of thoracic respiration with pronounced abdominal breathing, profuse perspiration, violent clonic and tonic convulsions, purposeless movements, and the extended period of time in which areflexia is present.

In some cases the reflexes may be absent for a period of two months or more. The case fatality rate is estimated to be about 10 per cent, and death is believed to be the result of respiratory paralysis.

The acute symptoms generally subside within ten days in the milder form. Khlentzos (1950) has reported sequelae consisting of dizziness, blurred vision, tremors of the hands, sensory changes in the legs, alopecia, interosseous atrophy, muscular weakness, ulnar palsy, foot drop, radial weakness, deviation of the tongue, atrophy of the tongue, and aphonia. In most instances these sequelae gradually disappeared within a period of two months.

Treatment: The treatment of moray eel poisoning is symptomatic. Paraldehyde is said to be the drug of choice in controlling the convulsions. Sodium amytal is not tolerated very well, although drop ether has given satisfactory results. The frequent changing, turning, aspiration of mucus, and convulsions prove to be a major nursing problem. The development of profuse mucus, trismus, and laryngeal paralysis may necessitate intubation. Atropine has been found to make the mucus more viscid and difficult to aspirate, and is not recommended. Nasal oxygen, antibiotics, whole blood, and intravenous fluids may be indicated. If cardiac failure is present digitalization may be required. In the cases reported by Ralls and Halstead (1955) intravenous injections of 1.5 to 2.5 gm. of 10 percent calcium gluconate and 10 per cent calcium chloride were administered and were found to give prompt relief in five out of six patients. One of the patients had a relapse within three hours requiring an additional injection of 2.0 gm. of calcium gluconate. The patient who was most severely intoxicated failed to respond to calcium gluconate therapy. There is no known specific antidote for the poison.

Prevention: Most of the outbreaks of moray eel poisoning have occurred in the tropical Pacific, although several Atlantic and Mediterranean species have been incriminated. If one is not absolutely certain as to the identification and the edibility of the species concerned, moray eels are best eliminated from the diet. Morays are reputed to be particularly dangerous to eat during their reproductive season, which probably occurs during the spring or early summer. At any rate, gonads and viscera of an eel should never be eaten. The old Biblical adage to eliminate scaleless and finless fish from one's diet has been continuously recommended by military survival manuals in regard to eating of fish in the tropics. It is good advice and should be followed.

Toxicology

There is no information available regarding the chemical or pharmacological properties of the toxin.

SCOMBROIDS

Order ACAUHOPTERYGII: Spiny-rayed fishes

Suborder SCOMBROIDEA: Mackerels, Skipjack, Tuna, etc.

The suborder Scombroidea includes four families: Scombridae, the true mackerels; Cybiidae, the Spanish mackerels; Katsuwonidae, the skip-jacks; and Thunnidae, the tunas. Scombroids are listed among man's most valuable food resources. However, under certain conditions they may cause intoxications.

Species:

Sarda sarda (Bloch) (Fig.) - Bonito.
Tropical and temperate Atlantic Ocean, and Mediterranean Sea.

Scomberomorus cavalla (Cuvier) (Fig.) - Sierra, kingfish,
Tropical Atlantic, Caribbean Sea. cavalla, pitado,
Spanish mackerel,
cero.

Scomberomorus maculatus (Mitchill) (Fig.)
East coast of America from Cape Ann to Brazil.

Scomberomorus regalis (Bloch) (Fig.) -
East coast of America, Cape Cod to Brazil.

Auxis thazard (Lacepede) (Fig.) - Frigate mackerel.
All warm seas.

Euthynnus alletteratus (Rafinescue) (Fig.) -
Circumtropical.

Katsuwonus pelamis (Linnaeus) (Fig.) - Oceanic bonito.
Circumtropical.

Pneumatophorus japonicus (Houttuyn) (Fig.) -
Warmer seas of Atlantic and Pacific Oceans, Mediterranean
Sea.

Pneumatophorus peruanus Jordan and Hubbs (Fig.) -
Costa Rica to Chile, Galapagos Islands.

Scomber scombrus Linnaeus (Fig.) - Common mackerel.
North Atlantic, south to Madeira and South Carolina, Baltic
and Mediterranean Seas.

Neothunnus macropterus (Temminck and Schlegel) (Fig.) -
Southern California to the Red Sea.

Thunnus alalunga (Bonnaterre) (Fig.) -
Circumtropical.

Thunnus thynnus (Linnaeus) (Fig.) - Tuna
Warm seas, circumtropical.

Mechanism of Intoxication

True scombroid poisoning is a unique form of ichthyosarcotoxism in which bacteria plays an important role in the production of the toxin due to inadequate preservation of the fish. However, the toxic agent is the result of bacterial action on certain non-toxic chemical substances which appear to be available in greater concentration in scombroids than most other types of fishes. Rarely fishes other than scombroids may be involved. It is believed that with the help of bacteria, a precursor, probably histidine, is converted to histamine by a process of decarboxylation. Other precursors may be converted to histamine-like products by the same process. Under normal circumstances histamine can be taken orally or enterally in large quantities without producing toxic symptoms. However, Geiger (1955) believes that in scombroid poisoning bacteria, or bacterial by-products, may either decrease the detoxification power or increase the absorptive ability of the intestinal mucosa. More recently Kawabata (1956) comment on his findings - date not available here. Kawabata et al 1955a & b not cited - text.

Medical Aspects

Clinical Characteristics: The symptoms of acute scombroid poisoning resemble those of histamine intoxication. The symptoms are characteristic, and appear with almost monotonous consistency. Frequently, toxic scombroid flesh can be detected immediately upon tasting it. Victims state that it has a "sharp or peppery" taste. Symptoms, usually developing within a few minutes after ingestion of the toxin, consist of intense headache, dizziness, throbbing of the carotid and temporal vessels, epigastric pain, burning of the throat, cardiac palpitation, rapid and weak pulse, dryness of the mouth, thirst, inability to swallow, nausea, vomiting, diarrhea, and abdominal pain. Within a short time a generalized erythema and an urticarial eruption may develop covering the entire body, accompanied by a severe pruritus. The face of the individual becomes swollen and flushed, the eyes become injected, and coryza develops. In severe cases there may be bronchospasm, suffocation, and severe respiratory distress. Various other minor discomforts such as fever, chills, malaise, tremors, metallic taste, and cyanosis of the lips, gums and tongue may occur. There is danger of shock, and deaths have been reported. However, the acute symptoms are generally transient, lasting only 8 to 12 hours. One usually severe case has been reported in which the acute symptoms continued for a period of 8 days.

If a poorly preserved neurotoxic scombroid fish is eaten, there may be a combination of both ciguatera and scombroid types of symptoms. In rare instances the clinical picture may become even more complex involving in addition to the two types of ichthyosarcotoxism one of the ordinary types of bacterial food poisoning.

Scombroid poisoning has also been termed tjakalang poisoning.

Treatment: There are very little data available specifically concerned with the treatment of scombroid poisoning. However, on the basis of the investigations of Legroux, Levaditi, Boudin and Bovet (1946ab), some recommendations are in order. These workers demonstrated that either histamine, or a closely allied product, is the cause of the intoxication. Moreover, it was found that antihistaminic drugs serve as an effective blocking agent in counteracting the symptoms of scombroid intoxication in laboratory animals. Goodman and Gilman (1955) recommend epinephrine as a specific physiological antagonist to histamine. Epinephrine will prevent or counteract all symptoms if administered promptly. If absorption of the toxic substance has occurred to any extent, antihistaminic drugs should be used to control the subsequent reaction. In the event of shock, intravenous fluids and plasma may be required. See also p. on the general treatment of ichthyosarcotoxism.

Prevention: Scombroid fishes appear to be particularly susceptible to putrefaction, and the development of toxic substances. Geiger (1944) has shown that the "histamine" content of the flesh of the Pacific mackerel, *Pneumatophorus diego*, increases from 0.00 mg. per 100 gm. of tissue about 95 mg. per 100 gm. when kept at room temperature (20 to 25°C) for about 10 hours. In warmer climates scombroids should be promptly refrigerated or eaten soon after capture. Toxic scombroid flesh cannot always be detected by appearance. The toxin content may be very high with little or no evidence of putrefaction. Scombroid meat having a sharp or peppery taste should be discarded. If the whole fish is available the gills should be examined. If there is the slightest evidence of putrefaction discard the fish.

Pharmacology and Chemistry

Scombroid poisoning:

The allergy-like symptoms which commonly follow the ingestion of certain types of fish which develop toxic properties post mortem were ascribed by early workers to the observed increase in histamine content of such fish (Geiger 1955). More recent data indicate that the active agent is not histamine but rather a histamine-like substance which possesses many of the pharmacological and physiological properties of histamine. The toxin resembles histamine in its allergy-like symptoms, in its vagus-stimulating properties, and in being counteracted by antihistaminic substances. Like histamine, it is a basic substance, is

dialyzable and precipitable with phosphotungstic acid. It differs from histamine in having a lesser solubility in alcohol, in being unstable to treatment with absolute alcohol and in having a different RF value. Kawabata, Ishizaka, and Miura (1955ab) have termed this substance "saurine". Histamine does not produce the observed symptoms from oral ingestion due to destruction in the digestive tract, unless the absorption mechanism of the intestine has first been damaged.

TETRAODONTOIDS

Order ACANTHOPTERYGII: Spiny-rayed Fishes (Cont.)

Suborder TETRAODONTOIDEA: Sunfishes, Sharp-nosed Puffers, Puffers, Porcupinefishes

The suborder Tetraodontoides is comprised of the families Molidae, the ocean sunfishes; Canthigasteridae, the sharp-nosed puffers; Lagocephalidae, Tetraodontidae, Colomesidae, Chonerhinidae, the puffers proper, and Diodontidae, the porcupinefishes. An outstanding characteristic of the group is the teeth, which are solidly united to form a beak. Spinous dorsal fin and pelvic fins are absent. Tetraodontoid fishes are scaleless.

The tetraodontoid fishes vary greatly in their size, habits and choice of habitat. The Molidae are the largest of the tetraodontoid fishes, members of the genus Mola attaining a length of more than three meters, and a weight in excess of three and one half tons. Sunfishes inhabit open seas, and are generally seen singly or in pairs, but seem to become gregarious at certain seasons when they band together in small schools which may consist of as many as a dozen individuals. During calm weather these fishes may be observed basking in the sun at the surface of the sea lying more or less on their sides.

The Canthigasteridae, or sharp-nosed puffers, are small brilliantly-colored marine fishes which abound in coral reefs and shoal areas. Most of the members of this family attain a maximum length of less than 13 cm.

The Colomesidae is comprised of a single species which inhabits the rivers of northern South America and the West Indies. Specimens have been taken from the mouth of the Amazon to the fringe of the Andes Mountains, a distance of more than three thousand miles from the nearest salt water.

The Chonerhinidae is a small family of freshwater fishes which, with the possible exception of a single African species, is restricted to the rivers of southern Asia and the Netherlands Indies. These fishes are said to attain a total length of about 28 cm. They are reputed to be very pugnacious and can inflict serious wounds with their sharp beak-like jaws.

The families Lagocephalidae and Tetraodontidae approximate each other in structure, appearance, and habits. Both families have marine, estuarine and freshwater representatives. Most lagocephalids range in size from about 25 to 50 cm., but some of the tetraodontids are larger. These puffers are generally considered to be shallow-water species, but they have been taken in large quantities many hundreds of miles from shore and at depths of 4000 fathoms or more.

A characteristic of all puffers is their remarkable ability to inflate themselves by gulping in large quantities of water, or air, if they are out of their fluid medium. Puffers make considerable noise during inflation by grinding their heavy jaw teeth together. Some species of puffers are covered with short prickly bristles which they appear to have the ability to withdraw or extend at will. Puffers have a distinctive offensive odor which is particularly noticeable when they are being dressed or dissected.

The Diodontidae, or porcupinefishes, are spiny marine fishes which are widely distributed in all warm seas. Most diodontids range in length from 20 to 50 cm., but Diodon hystrix may attain a total length of 91 cm. or more. Porcupinefishes inhabit coral reefs and shoal areas, generally traveling singly or in pairs. They also have the ability to inflate themselves until they are almost spherical in outline. During the inflation process the spines, which are usually depressed, are extended, giving the fish a formidable appearance.

Because of the larger number of species of tetraodontoids and their close resemblance to each other in appearance, only a few representative species are listed below.

Species: Canthigasteridae

Canthigaster bennetti (Bleeker) (Fig.) - Sharp-nosed puffer.
East Africa, Netherlands Indies, Australia, Micronesia, Polynesia.

Canthigaster rivulatus (Temminck and Schlegel) (Fig.) -
Sharped-nosed puffer. Hawaiian Islands, Japan.

Chonerinidae

Zonopterus naritus (Richardson) (Fig.) - Puffer
Rivers of Burma, Sumatra, Borneo, Sea of Penang.

Colomesidae

Colomesus politacus (Bloch and Schneider) (Fig.) -
Freshwater puffer.

Diodontidae

Chilomycterus orbicularis (Bloch) (Fig.) - Porcupinefish,
Spiny Blaesop. Cape of Good Hope and tropical Indian
Ocean, Netherlands Indies, Philippine Islands.

Diodon hystrix (Linnaeus) (Fig.) - Porcupinefish
All tropical seas, north to Lower California, Hawaiian
Islands, and Florida.

Lagocephalidae (Puffer, Swelltoad,
toadfish, Globefish,
Swellfish, Rabbitfish,
Ppote, Fugu)

Amblyrhynchotes honckenii (Bloch) (Fig.) -
South Africa, Netherlands Indies, China

Fugu chrysops (Hilgendorf) (Fig.) -
Pacific coast of central Japan.

Fugu ocellatus ocellatus (Linnaeus) (Fig.) -
China, Japan, Philippine Islands.

Fugu pardalis (Temminck and Schlegel) (Fig.) -
China, Japan

Fugu poecilonotus (Temminck and Schlegel) (Fig.) -
South Africa, Malay Archipelago, China, Korea, Japan.

Fugu rubripes (Temminck and Schlegel) (Fig.) -
China, Korea, Japan.

Fugu stictonotus (Temminck and Schlegel) (Fig.) -
Southern Korea, East China Sea and adjoining waters,
Japan.

Fugu vermicularis porphyreus (Temminck and Schlegel) (Fig.)
East China Sea and adjoining waters, Japan.

Fugu vermicularis vermicularis (Temminck and Schlegel)
(Fig.) - East China Sea, Japan.

Lagocephalus laevigatus inermis (Temminck and Schlegel)
East Africa, tropical Indian Ocean, Australia, East
China Sea, southern Japan.

Sphaeroides annulatus (Jenyns) (Fig.)
California to Peru, Galapagos Islands

Sphaeroides maculatus (Bloch and Schneider) (Fig.)
Atlantic coast of United States to Guiana.

Sphaeroides testudineus (Linnaeus) (Fig.)
Atlantic coast of United States, West Indies, Brazil.

Torquigener hamiltoni (Gray and Richardson) (Fig.)
Australia, Melanesia, Polynesia.

Holidae (Ocean sunfish, headfish, king of
the machereis)

Mola mola (Linnaeus) (Fig.)
Temperate and tropical seas.

Ranzania laevis (Pennant) (Fig.)
All temperate and tropical seas.

Tetraodontidae (See Lagocephalidae for
common names)

Arothron hispidus (Linnaeus) (Fig.)
Polynesia, Micronesia, Melanesia, Australia, Japan, to the
Red Sea and South Africa.

Arothron meleagris (Lacepede) (Fig.)
Netherlands Indies through the tropical Pacific to western
coast of Central America.

Arothron nigropunctatus (Bloch and Schneider) (Fig.)
Tropical Indo-Pacific, Red Sea and East Africa to southern
Japan, Australia, Melanesia, Micronesia, Polynesia.

Chelonodon patoca (Hamilton and Buchanan) (Fig.)
Southeast Africa, India, Netherlands Indies, Australia,
China, Ryukyu Islands.

Dichomycter fluviatilis (Hamilton and Buchanan) (Fig.)
Freshwaters and coasts of India, Burma, Malay Peninsula,
Netherlands Indies.

Tetraodon lineatus (Linnaeus) (Fig.)
Rivers of northern and western Africa.

Medical Aspects

Clinical Characteristics: The onset and symptoms in puffer poisoning vary according to the person and the amount of poison ingested. However, malaise, pallor, dizziness, paresthesias of the lips and tongue, ataxia, most frequently develop within 10 to 45 minutes after ingestion of the fish, but cases have been reported in which the symptoms did not develop for three hours or more. The paresthesias which the victim usually describes as a "tingling or prickling sensation" may subsequently involve the fingers and toes, then spread to other portions of the extremities, and gradually develop into severe numbness. In some cases the numbness may involve the entire body, in which instances the patients have stated that it felt as though their bodies were "floating". Hypersalivation, profuse sweating, extreme weakness, precordial pain, headache, subnormal temperatures, decreased blood pressure and a rapid weak pulse usually appear early in the succession of symptoms. Gastro-intestinal symptoms of nausea, vomiting, diarrhea and epigastric pain are sometimes present early in the disease, whereas in other cases they are totally lacking. Contradictory statements appear in the literature relative to pupillary changes, but these differences can probably be resolved on the basis of the time at which the examination is made. Apparently the pupils are constricted during the initial stage and later become dilated. As the disease progresses the eyes become fixed and the pupillary and corneal reflexes are lost.

Shortly after the development of the paresthesias, respiratory symptoms become a prominent part of the clinical picture. Respiratory distress, increased rate of respiration, movements of the nostrils, and a diminution in depth of respiration are generally observed. Respiratory distress later becomes very pronounced, and the lips, extremities and body become intensely cyanotic. Petechial hemorrhages involving extensive areas of the body, blistering and subsequent desquamation have been reported. Severe hematemesis has also been known to occur. Muscular twitching, tremor and incoordination become progressively worse and finally terminate in an extensive muscular paralysis. The first areas to become paralyzed are usually the throat and larynx, resulting in aphonia, dysphagia and later complete aphagia. The muscles of the extremities become paralyzed and the patient is unable to move. As the end approaches the eyes of the patient become fixed and glassy, and convulsions may occur. The victim may become comatose, but in most instances they retain consciousness and their mental faculties remain acute until shortly before death.

Two interesting examples of the comatose condition in puffer poisoning have been cited by Akashi (1880). A gambler "died" from eating fugu, and the body was placed in storage for officials to examine. About seven days later the man became conscious, and finally recovered. The victim claimed to have recalled the entire incident,

and stated that he was afraid he would be buried alive. In the second case the victim was considered to be dead, and was placed on a cart and shipped to a crematorium in a nearby town. The man recovered as he was being removed from the cart, and walked away. This latter victim also claimed to be aware of what was happening.

Death results from a progressive ascending paralysis involving the respiratory muscles. On the basis of the Japanese statistics the case fatality rate is 61.5 per cent. Puffer poisoning is the greatest single cause of fatal food intoxications in Japan. If death occurs, it generally takes place within the first six hours, or within 24 hours at the latest. Death may occur within 17 minutes after ingestion of the fish. The prognosis is said to be good if the patient survives for 24 hours. There are no data available regarding the laboratory findings of tetraodon poisoning.

It has been said that the poison may be absorbed through the skin by simple contact with the fish. The handling of puffer viscera over several days has been known to produce malaise, intense headache, itching and rash on the face and hands, and gastro-intestinal upset.

Treatment: The treatment of tetraodon poisoning is purely symptomatic. There is no known antidote for tetraodontoxin. It is essential to remove the poison from the body as rapidly as possible. Ingestion of large quantities of sodium bicarbonate solution has been recommended as efficacious in attenuating the effects of the toxin, and as an emetic. Any of the ordinary emetics are helpful, but apomorphine is said to yield the best results. Laxatives and enemas have also been found to be useful. As the disease progresses the physician must direct his main efforts toward combating respiratory distress and the accompanying drop in blood pressure. Although artificial respiration may prolong life for a brief period there is no indication that it will prevent death in severe intoxications. There is little other data of value in the treatment of this disease.

Prevention: If one eliminates scaleless fish from the diet there is no opportunity to come in contact with tetraodontids. The eating of puffer or fugu, as it is termed in Japanese, constitutes an important public health problem in Japan. If a person is living in Japan and has a desire to eat puffer, he should purchase the fish from a first class authorized restaurant having a licensed puffer cook. It is important that the individual preparing the food has a thorough knowledge regarding the fish and its toxicity, and the relative toxicity of the various organs of the puffer. One can generally have this assurance by dealing only with the higher class restaurants. It is advisable to eliminate all of the visceral organs and skin from the diet regardless of the species. Although the testes are usually non-toxic, it must be kept in mind that this organ is frequently confused with the violently toxic ovaries, particularly during the season of the year in which the reproductive organs are in their dormant state.

Cooking by frying, stewing, baking, boiling, etc., does not inactivate the toxin. The toxin may still be present in lethal quantities even when passed through a steam retort in the commercial canning process. The poison can be inactivated chemically by cooking the meat in a strong solution of sodium bicarbonate, ordinary baking soda, for a prolonged period of time. This latter technique also destroys the flavor of the fish and renders it useless for consumption.

Our knowledge regarding the toxicity of most species of tetraodontoid fishes, exclusive of Japanese forms, is exceedingly meager. Many of the tropical tetraodontoid species have produced violent death at one time or another. Unless a species is definitely known to be non-toxic, which is questionable even under the best of circumstances, all tropical forms should be eliminated from the diet.

If it is a question of survival--either eat the fish or die--and the edibility of the fish is unknown, it is recommended that the fish be eviscerated promptly, and only the musculature be used. Moreover, the meat should be cut or torn into small bits, and soaked in water for a minimum of three or four hours. During this period the flesh should be kneaded while in the water, and the water changed at frequent intervals. The toxin is water-soluble and leaching will effectively remove it.

Pharmacology

The physiological effects of puffer poison have been studied, principally by Japanese investigators, since the time of Osawa and Furukawa (1885). The most comprehensive single work on the history of poisonous puffer investigation is that of Suehira (1947). Other very useful reviews of the physiology of puffer poison are those of Yudkin (1945), Couillard (1955) and Goe (1957).

The poison of the puffer appears to be primarily a neurotoxin. Whether or not it has a special predilection for certain kinds of nervous tissue or certain portions of the nervous system has not been fully established. Death from puffer poison in most experimental animals is usually the result of respiratory stasis. The relative importance of the poisoning of the medullary center, the peripheral nerves or the respiratory muscles in the poisoning sequence is not known. Experimental evidence indicates that central synapses, peripheral axones, and skeletal muscle may all be inhibited by the toxin. In addition, puffer toxin is known to interfere with neuro-muscular transmission first by raising the threshold of the muscle fiber and secondly blocking the development of the end plate potential. The respiratory arrest may or may not be accompanied by convulsions, depending upon the experimental animal.

Conduction in the spinal cord (at least transverse conduction and probably also longitudinal) is impaired by puffer poison. This results in an impairment of most of the reflex activity involving the spinal cord.

Hypotension is usually concomitant with the poisoning of respiration but again the relative importance of the medullary centers, the peripheral ganglia, or the blood vessel musculature in the sequence is not known.

A cardio-inhibitory action that results in paralysis first of the musculature, next of the conduction system and lastly of the pacemaker seems to be well established.

Chemistry

No investigator has as yet ventured to publish a suggested structure for puffer toxin and even the empirical formula cannot be considered as firmly established at this time. There are two forms of the toxin, one having a toxicity approximately double that of the other. The more potent form is convertible to the less potent form by heating in water or dilute acid under controlled conditions or by addition of alcohol and much ether to an acetic acid solution of the toxin. The conversion is reversible by addition of alcohol but not ether to an acetic acid solution of the toxin and crystallizing under refrigeration.

The analytical data for the less potent form are in agreement with the formula $C_{11}H_{19}O_8N_3$ while that of the more potent form

favors the formula $C_{12}H_{17}O_{10}N_3$. The infrared spectrograms and the paper chromatograms are identical for both forms and indicate the presence of OH (or NH) groups and amide carbonyl (or C-N) groups. Two double bonds are present which are probably those of two acid amide groups. The toxin molecule does not contain any ester, lactone, lactan, ketone or aldehyde type carbonyl groups. Since the toxin forms salts readily with acids, the third nitrogen atom may be considered as representing an amine group which is probably a secondary amine (Tsuda and Kawamura-1952, 1953; Yokoo-1950; Yokoo and Morosawa-1955).

CIGUATERA-PRODUCING FISHES

Order ACANTHOPTERYGII: Spiny-rayed Fishes (Cont.)

Ciguatera-producing fishes are widely distributed throughout the Acanthopterygii. The etiological agents of this disease are marine fishes, and generally shore forms, although oceanic species are sometimes incriminated. In terms of numbers of species involved, over-all public health and economic significance, it is probably the most important form of ichthyo-sarcotoxism. The great importance of the disease is not based on mortality statistics, but rather its unique unpredictability, striking at the most unexpected times and places, thereby affecting local fisheries resources in an adverse manner and constituting a public health problem of considerable magnitude.

Ciguatera was first reported in 1530 in the West Indies, and in the Pacific area in 1658. Since this early period several hundred species of marine fishes have been incriminated as the causative agents of the ciguatera type of fish poisoning. Apparently, any marine fish under certain circumstances may become poisonous as a result of its food habits. An edible and commercially valuable species in one area may be deadly poisonous in another. The edibility of fishes in an island area may suddenly change. Ciguatera intoxications first began to appear in the islands of Midway, Johnston, Palmyra, Fanning, and Christmas about the year 1943 as a result of eating fishes which had been previously known to be edible.

Because of the large number of fishes which have been incriminated as toxic it is not feasible to list all of them, hence only some of the more common representative species are listed below.

Species:

Acanthuridae - Surgeonfishes.

Acanthurus achilles (Shaw)

China, Philippines, Hawaiian Islands

Acanthurus glaucopareius (Cuvier)

East Indies, Philippine Islands, Oceania, and the tropical eastern Pacific.

Acanthurus triostegus (Linnaeus)

Hawaiian Islands, and Johnston Island.

Zebrasoma veliferum (Bloch)

Indo-Pacific, Red Sea.

Albulidae - Ladyfishes

Albula vulpes (Linnaeus)

Hawaiian Islands, Red Sea.

Aluteridae - Filefishes

Alutera monoceros (Linnaeus)

All warm seas.

Pervagor timentosus (Linnaeus)

East Indies, China, Queensland, Micronesia, Polynesia.

Balistidae - Triggerfishes

Balistes vetula (Linnaeus)

Tropical Atlantic, Mediterranean, and Indian Ocean.

Balistoides niger (Bonnaterre)

Madagascar to the Philippines, China, Japan, Formosa,
Melanesia, Micronesia, Polynesia.

Melichthys buni (Lacepede)

Indo-Pacific, from the Seychelles and Philippines to
the Hawaiian Islands, and the islands off the Pacific
coast of Mexico.

Pseudobalistes flavomarginatus (Ruppell)

Red Sea, Indian Ocean, tropical Pacific

Rhinecanthus aculeatus (Linnaeus)

Indo-Pacific.

Belonidae - Houndfish

Strongylura caribbaea (Lesueur)

West Indies

Bothidae - Brill, Turbot

Scophthalmus rhombus (Linnaeus)

Coasts of Europe, from Scandinavia to the Mediterranean.

Carangidae - Jacks, Cavallas, Pampanos

Caranx bartholomaei (Cuvier)

Caribbean, West Indies, northward to Florida and
North Carolina.

Caranx hippos (Linnaeus)

Tropical Atlantic.

Caranx latus (Agassiz)

Tropical western Atlantic.

Caranx lugubris (Poey)

Circumtropical.

Caranx melampygus (Cuvier)

Tropical Indo-Pacific to the Philippines, Japan,
Marcus Island, Hawaiian Islands, Society, and
Marquesas Islands.

Caranx sefasciatus (Quoy and Gaimard)

Indo-Pacific

Elagatis bipinnulatus (Quoy and Gaimard)
Circumtropical.

Scomberoidea sanctipetri (Cuvier)
Polynesia, Australia, Indo-Pacific area, east Africa,
Red Sea.

Seriola falcata (Valenciennes)
West Indies, north to Florida and the Carolinas.

Trachurops crumenophthalmus (Bloch)
Circumtropical.

Trachurus trachurus (Linnaeus) - Horse mackerel
North Atlantic, chiefly on the northern coast of Europe,
south to Spain and Mediterranean.

Chaetodontidae - Butterflyfishes

Chaetodon reticulatus (Cuvier)
Polynesia, India, East Indies, China, Japan, Micronesia.

Pygoplites diacanthus (Boddaert)
Polynesia, Micronesia, Melanesia, East Indies, Mauritius,
Zanzibar, Red Sea.

Clupeidae - Shad, Sprat, Herring

Alosa alosa (Linnaeus)
Northern parts of Eastern Atlantic.

Anodontostoma chacunda (Hamilton-Buchanan)
Philippines, East Indies, Hainan, New Guinea and west to
India and Mauritius.

Clupanodon thrissa (Linnaeus)
Polynesia, Philippines, China, Formosa, Korea, East Indies,
Japan, India.

Clupea sprattus (Linnaeus)
Northeastern Atlantic, Mediterranean.

Clupea tropica (Osbeck)
Seychelles Islands, Coast of Africa.

Harengula humeralis (Cuvier)
Southern shores of the United States to Brazil.

Harengula ovalis (Bennett)
Indo-Pacific, Red Sea.

Macrura ilisha (Hamilton-Buchanan)
Persian Gulf, India, Ceylon, Burma.

Nematalosa nasus (Bloch)
Australia, Philippines, East Indies, India, Burma.

Opisthonema oglinum (Lesueur)
West Indies.

Sardinia pichardus (Walbaum)
Eastern Atlantic from the Canaries northward to the British Isles.

Sardinella fimbriata (Valenciennes)
Philippines, East Indies, Hainan, China.

Coryphaenidae - Dolphin

Coryphaena hiopurus (Linnaeus)
All tropical and temperate seas.

Dussumieridae - Round Herring

Dussumieria acuta (Valenciennes)
China, Philippines, East Indies, Malaya, India, Red Sea.

Engraulidae - Anchovies

Engraulis japonicus (Schlegel)
China, Japan, Korea, Formosa.

Gobiidae - Gobies

Gtanogobius criniger (Valenciennes)
Zanzibar to the Philippines, and Palau Islands, and south through the East Indies to the New Hebrides and Australia.

Hemirhamphidae - Halfbeaks

Hemirhamphus brasiliensis (Linnaeus)
Tropical Atlantic.

Holocentridae - Squirrelfishes

Myripristis murdian (Forsk.)
Indo-Pacific.

Katsuwonidae - Bonito

Kathynnus vaito (Kishinouye)
Tropical Pacific

Katsuwonus pelamis (Linnaeus)
Circumtropical

Labridae - Wrasse

Bodianus rufus (Linnaeus)
Florida and West Indies, southward to Brazil.

Coris gaimardi (Quoy and Gaimard)
Tropical Indo-Pacific, from Zanzibar to Ryukyus, Micronesia,
Polynesia.

Epibulus insidiator (Pallas)
Tropical Indo-Pacific.

Lutjanidae - Snappers

Aphareus furcatus (Lacepede)
Cocos Island.

Aprion virescens (Valenciennes)
Tropical Indo-Pacific.

Gnathodentex aureolineatus (Lacepede)
Tuamotu Archipelago westward to East Africa.

Lethrinus miniatus (Forster)
Polynesia, Japan, westward to east Africa.

Lethrinus mambo (Montrouzier)
Melanesia.

Lethrinus variagatus (Valenciennes)
Tropical Indo-Pacific.

Lutjanus bohar (Forskol)
Polynesia, Melanesia, Philippines, east Africa, Red Sea.

Lutjanus gibbus (Forskol)
Tropical Indo-Pacific.

Lutjanus monostigma (Cuvier)
Polynesia, Melanesia, Micronesia, China, East Indies,
Seychelles, Madagascar, Red Sea.

Lutjanus vaigiensis (Quoy and Gaimard)

Polynesia, Japan, Australia, westward to east Africa.

Monotaxis grandoculis (Forskol)

Polynesia westward to east Africa.

Pardicichthys venenatus (Whitley)

Australia.

Mullidae - Goatfish, Surmulletts

Parupeneus chryserydros (Lacepede)

Polynesia westward to East Africa.

Upeneus ~~large~~ (Jordan and Evermann)

Polynesia, Micronesia.

Ostraciontidae - Trunkfish

Lactophrys trigonus (Linnaeus)

Atlantic coast of tropical America, northward to Cape Cod.

Lactoria diaphanus (Bloch and Schneider)

Tropical Indo-Pacific, and eastern Pacific from California south.

Lactoria cornutus (Linnaeus)

Tropical Indo-Pacific.

Ostracion cubicus (Linnaeus)

Tropical Indo-Pacific.

Rhinesomus gibbosus (Linnaeus)

Indo-Pacific, Japan, westward to Zanzibar, Red Sea, and Aden.

Pleuronectidae - Flounders

Platichthys flesus (Linnaeus)

Coasts of Europe, from the White Sea to the Black Sea, entering freshwater.

Pomacentridae - Damselfishes

Abudefduf septemfasciatus (Cuvier)

Polynesia, China, westward to east Africa.

Scaridae - Parrotfishes

Scarus caeruleus (Bloch)

Florida and West Indies.

Scarus ghobban (Forskol)

Polynesia, China, westward to Red Sea.

Scarus microrhinos (Bleeker)

Indo-Pacific.

Scarus vetula (Bloch and Schneider)

West Indies to Florida.

Scatophagidae - Spadefish

Scatophagus argus (Linnaeus)

Philippine Islands, East Indies and China, west to India and Ceylon, southeast to the New Hebrides, New Caledonia and Australia, in the sea, rivers and lakes.

Serranidae - Seabasses, Groupers

Cephalopholis argus (Bloch and Schneider)

Tropical Indo-Pacific

Epinephelus adscensionis (Osbeck)

Florida and West Indies southward to Brazil.

Epinephelus fuscoguttatus (Forskol)

Indo-Pacific.

Epinephelus guttatus (Linnaeus)

South Carolina to Brazil.

Epinephelus maculatus (Bloch)

Polynesia, China, to east Africa.

Mycteroperca venenosa (Linnaeus)

Western tropical Atlantic.

Paranthias furcifer (Valenciennes)

Cuba to Brazil.

Plectropomus truncatus (Fowler)

Micronesia, East Indies, Philippines.

Rypticus saponaceus bicolor (Valenciennes)

Baja California to the Galapagos Islands and northern Peru.

Variola louti (Forskol)

Tropical Indo-Pacific.

Siganidae - Rabbitfishes

Siganus lineatus (Valenciennes)

Philippine Islands, Santa Cruz Islands, New Guinea, Solomon Islands, Australia; and Okinawa.

Sparidae - Porgies, Bream

Calamus calamus (Valenciennes)
Florida and West Indies.

Pagellus erythrinus (Linnaeus)
Black Sea, Mediterranean, and east Atlantic, from the British Isles and Scandinavia to the Azores, Canaries and Fernando Po.

Pagrus pagrus (Linnaeus)
Eastern Atlantic, Mediterranean Sea.

Sparus sarba (Forskol)
Indo-Pacific, westward to the Red Sea.

Sphyraenidae - Barracudas

Sphyraena barracuda (Walbaum)
Indo-Pacific, from Hawaii to the Red Sea, Madagascar; West Atlantic from Brazil to the West Indies, Florida and Bermuda.

Tetragonuridae - Squaretails

Tetragonurus cuvieri (Risso)
Temperate regions of the world.

Medical Aspects

The clinical recognition of ciguatera has been much confused with other disease entities. It is most frequently classed as a form of bacterial food poisoning, with which it has no etiological connection. Ciguatera or the ciguatera type of ichthyosarcotoxism has been also designated as ciguatos, ciguabo, siguatera, and the Pacific type of ichthyosarcotoxism.

Clinical Characteristics: The time of onset and symptomatology varies greatly according to the person, species of fish, toxicity, amount and portion of fish ingested. Tingling about the lips, tongue, and throat followed by numbness may develop immediately or any time within a period of 30 hours after the ingestion of the toxin. The usual time interval for the development of symptoms is from one to six hours. The initial symptoms in some instances consist of nausea, vomiting, metallic taste, dryness of the mouth, abdominal cramps, tenesmus and diarrhea, followed by perioral tingling and numbness. The muscles of the mouth, cheeks, and jaws may become drawn and spastic with a feeling of numbness. Generalized symptoms of headache, anxiety, malaise, prostration, dizziness, pallor, cyanosis, insomnia, chilly sensations, fever, profuse sweating, rapid weak pulse, weight loss,

myalgia, and joint aches are frequently present. Victims usually complain of a feeling of profound exhaustion and weakness. The feeling of weakness may become progressively worse until the patient is unable to walk. Muscle pains are generally described as a dull, heavy ache, or cramping sensation, but on occasion may be sharp, shooting, and affect particularly the arms and legs. Victims complain of their teeth feeling loose and painful in their sockets. Visual disturbances, consisting of blurring, temporary blindness, photophobia, and scotoma are common. Pupils are usually dilated and the reflexes diminished. Skin disorders are frequently reported, which are generally initiated by an intense generalized pruritus, followed by erythema, maculopapular eruptions, blisters, extensive areas of desquamation - particularly of the hands and feet - and occasionally ulceration. There may also be a loss of hair and nails.

In severe intoxications the neurotoxic components are especially pronounced. Paresthesias involve the extremities, and paradoxical sensory disturbances may be present in which the victim interprets cold as a "tingling, burning, dry-ice or electric-shock sensation," or hot objects may give a feeling of cold. In regard to the paradoxical sensory disturbance, the classical case is usually cited in which an American naval officer who was poisoned by eating an amberjack was later observed subconsciously blowing on his ice cream, which was "burning his tongue," in order to cool it. Ataxia and generalized motor incoordination become progressively worse. The reflexes are diminished. Muscular paralyses develop. There may be clonic and tonic convulsions, muscular twitchings, tremors, dysphonia, dysphagia, coma, and death by respiratory paralysis. The limited morbidity statistics show a case fatality rate of about 7 per cent. Death may occur within 10 minutes, but generally requires several days.

In those instances in which the victim survives, recovery is slow and may be very prolonged, with extreme weakness, sensory disturbances, and excessive weight loss being the last symptoms to disappear. Severe intoxications have occurred in which the patient survived, but complete recovery required a period of several years. Several workers have reported cases in which the symptoms may persist for as long as 25 years. Individuals who have been severely intoxicated have stated that during periods of stress, fatigue, exposure or poor nutrition, there is a recurrence of the myalgia and joint aches similar to that suffered during the original acute period of the disease.

Treatment: An attack of fish poisoning does not impart immunity and there is no known specific antidote. The treatment is purely symptomatic. Gastric lavage and catharsis should be instituted at the earliest possible time. In many instances 10 per cent calcium gluconate given intravenously has given prompt relief, whereas in others it has been ineffective. Paraldehyde and ether inhalations

have been reported to be effective in controlling the convulsions. Nikethamide or one of the other respiratory stimulants is advisable in cases of respiratory depression. In patients where excessive production of mucus is a factor, aspiration and constant turning are essential. Atropine has been found to make the mucus more viscid and difficult to aspirate, and is not recommended. If laryngeal spasm is present, intubation and tracheotomy may be necessary. Oxygen by inhalation and intravenous administration of fluids supplemented with vitamins given parenterally are usually beneficial. If the pain is severe, opiates will probably be required. Morphine given in small divided doses has been suggested. Cool showers have been found to be effective in relieving the severe itching. Fluids given to patients suffering from the paradoxical sensory disturbance should be slightly warm or at room temperature. Vitamin B complex supplements are advisable.

Prevention: One cannot detect a poisonous fish by its appearance. Moreover, there is no known simple chemical test to determine the edibility of a fish. The most reliable methods involve the preparation of tissue extracts which are injected intraperitoneally into mice, or feeding samples of the viscera and flesh to cats, or dogs, and observing the animals for the development of toxic symptoms. Viscera, i.e., liver and intestines, of tropical marine fishes should never be eaten. Also, the roe of most marine fishes is potentially dangerous, and in some cases may produce rapid death. Fishes which are usually large for their size should be discarded, particularly barracuda (Sphyraena), jack (Caranx), and grouper (Epinephelus), during their reproductive season. If one is living under survival conditions and questionable fishes must be eaten, it is advisable to cut the fish into thin fillets and soak them in several changes of water for at least 30 minutes. Do not use the rinse water for cooking purposes. This will serve to leach out the poison which is readily water-soluble. If a questionable species is cooked by boiling, the water should always be discarded. Again, it should be pointed out that ordinary cooking procedures do not attenuate ichthyosarcotoxins. The advice of aboriginal people regarding the eating of tropical marine fishes is frequently conflicting and erroneous.

Chemistry

All chemical work to date on the agent responsible for ciguatera intoxication(s) has been of a preliminary nature, and no serious, long range program in the past has been instituted with the idea of defining the toxin's chemical and physical characteristics. Hiyama (1943, 1950) in his extensive report on poisonous fishes of the South Seas, showed that ciguatera can be extracted from fish with water or alcohol, and that the toxin is heat stable. As an indication that a small molecular species is being dealt with, Halstead and Ralls (1954) dialyzed an aqueous solution of ciguatera toxin and found a measurable toxicity in the dialyzate.

Hashimoto (1956) has reported on the chemical nature of the toxin present in a barrusuda Sphyræna picuda, which had poisoned 30 persons in Tokyo in 1949. He corroborated the alcohol and water solubility of the crude toxin and also showed that the poison was soluble in ether and acetone, but insoluble in water acidified with tartaric acid. The ether and acetone solubilities strongly suggest that the toxic agent is not the same as paralytic shellfish and puffer poisons.

Exclusive of the preceding meager data, nothing is known about the chemical and physical characteristics of ciguatera. Its melting and/or boiling point, elementary analysis, molecular weight, optical and spectral properties, molecular structure, synthesis, etc. remain as problems for future investigations.

GEMPYLIDS

Order ACANTHOPTERYGII: Spiny-rayed Fishes (Cont.)

The gempylids, escolar, or pelagic mackerels, are a small group of predacious oceanic fishes. They have a band-shaped body and large sharp teeth. There are two dorsal fins present, the first of which is spinous and longer than the second. They are distinguished from the true mackerel by the complete absence of a lateral keel or ridge on the caudal peduncle. Gempylids produce an oil which has a pronounced purgative effect.

Species:

Lepidocybium flavobrunneum (Smith) (Fig.) - Snake mackerel, escolar. California, Hawaiian Islands, Japan, Australia, Africa.

Ruvettus pretiosus (Cocco)
Warmer parts of Atlantic and Indo-Pacific.

Medical Aspects

The effects of ingesting the oil from gempylids can hardly be considered as an intoxication in the usual sense of the word. The purgative effect is usually obtained from ingesting the laxative-like oil, which is particularly abundant in the marrow of the bones and in the rich oily flesh. Diarrhea develops rapidly, and is without cramping or pain. No other untoward effects have been reported from ingesting gempylid oil.

ICHTHYOTOXIC FISHES

Included within this section is a group of freshwater, anadromous, or brackish-water fishes. Since this form of fish poisoning results from ingesting a toxin which appears to be restricted to the gonads of certain freshwater and a few marine species the disease is designated as ichthyotoxism.

LIST OF FRESHWATER FISHES REPORTED AS POISONOUS TO EAT

Order ACIPENSEROIDEI: Sturgeons

Species:

Acipenseridae

Acipenser huso (Linnaeus)

Black Sea, Sea of Azov, Caspian Sea and the Mediterranean Sea, and the Rivers that drain into these seas.

Acipenser sturio (Linnaeus)

Both coasts of the Atlantic and the Mediterranean Sea.

Order OSTARIOPHYSI: Catfishes

Species:

Ariidae

Selenaspis herzberg (Bloch)

South America from the Amazon northward.

Pseudobagrus aurantiacus (Temminck and Schlegel)

Japan.

Cottidae: Sculpins

Scorpaenichthys marmoratus (Ayres) (Fig.) - Cabezon

Pacific coast of North America.

Cyprinidae: Minnows

Barbus barbus (Linnaeus) (Fig.) - Barbel

Northern and Central Europe.

Schizothorax intermedius (McClelland) (Fig.)

Central Asia.

Tinca tinca (Linnaeus) (Fig.) - Tench

Europe.

Gadidae: Burbot

Lota lota (Linnaeus) (Fig.) - Burbot, Eel Pout

Freshwaters of northern and central Europe.

Medical Aspects

Numerous clinical reports have appeared in European literature relative to fish poisoning resulting from the ingestion of the roe of certain fishes. Toxic species are most dangerous during the height of their gonadal activity, usually March through June. Symptoms develop soon after ingestion of the roe, and consist of abdominal pain, nausea, vomiting, diarrhea, bitter taste, dryness of the mouth, intense thirst, cold sweats, rapid irregular weak pulse, pupillary dilatation, syncope, chest pain, pallor, dysphagia, and tinnitus. In severe cases there may be muscular cramps, convulsions and coma. Deaths have occurred in rare instances, particularly from Schizothoras. However, in most instances people recover from ichthyotoxism within a period of five days. Treatment is symptomatic.

ICHTHYCHAENOTOXIC FISHES

Intoxication resulting from the ingestion of fresh fish blood is too rare to be considered of military significance and will therefore receive no further attention.

LITERATURE CITED

- | | |
|--|--|
| Akashi, T.
1880 | Experiences with fugu poisoning. Iji Shimbun, (27): 19-23. (In Japanese). |
| Autenrieth, H. F.
1833 | Ueber das Gift der Fische. C. F. Oslander, Tübingen: 1-287. |
| Baje, O.
1939 | Toxin in the flesh of the Greenland shark. Meddel. om Grønland, 125 (5): 1-16 |
| Cohen, S. C.,
J. T. Emert
and F. C. Goss
1946 | Poisoning by barracuda-like fish in the Marianas. U. S. Nav. Med. Bull., 46 (2): 311-317, 2 figs., 2 tabs. |
| Couillard, P.
1955 | Anticoagulant and antimitotic substances from the ovary of the puffer, <u>Spheroides maculatus</u> , (Bloch and Schneider). Thesis, PH. D., Univ. of Pennsylvania: 1-85. |
| Coutaud, H.
1879 | Observations sur sept cas d'empoisonnement par le foie de requin à l'île des Pins (Nouvelle Calédonie) en 1873. Thesis, Montpellier, (8): 9-47. |
| Engelsen, H.
1922 | Om giftfisk og giftige fisk. Nord. Hyg. Tidskr., 3: 316-325. |

- Fish, C. J., and H. C. Cobb
1954 Noxious marine animals of the central and western Pacific Ocean. Res. Rept. U. S. Fish and Wildlife Serv., (36): 1-45, 3 figs.
- Fonsagrives, J. B.
1877 Traite d'hygiene navale. 2nd ed. Bailliere et Fils, Paris: 620-650, 11 figs.
- Fonsagrives, J. B., and L. de Mericourt.
1861 Recherches sur les poissons toxicophores exotiques des pays chauds. Ann. Hyg. Pub. Med. Leg., Paris, ser. 2. 16 (2): 326-359.
- Geiger, E.
1944 Histamine content of unprocessed and canned fish. A tentative method of quantitative determination of spoilage. Food Res., 9(4): 293-297.
- Geiger, E.
1955 Role of histamine in poisoning with spoiled fish. Science, 121 (3155), 865-866.
- Gilman, R. L.
1942 A review of fish poisoning in the Puerto Rico-Virgin Islands area. U. S. Nav. Med. Bull., 40 (1): 19-27, 2 pls.
- Goe, D. R.
1957 The neurophysiological action of extracts from the gulf puffer, Sphoeroides angulatus. Doctoral dissertation. University of Southern California.
- Goodman, L. S. and A. Gilman
1955 The pharmacological basis of therapeutics. 2nd ed. Macmillan Co., New York: 1-1831, 89 figs., 76 tabs.
- Gregory, C. R.
1925 Three cases of food poisoning attributed to eating freshly caught fish. U. S. Nav. Med. Bull., 23 (3): 316-320.
- Halstead, B. W.
1951a Poisonous fish - a medical-military problem. Res. Rev., U. S. Navy, NAVEXOS P-510:10-16, 8 figs.
- Halstead, B. W.
1951b Ichthyotoxism, a neglected medical problem! Med. Arts Sci., 5(4): 115-121, 8 figs.
- Halstead, B. W., and W. N. Lively
1954 Poisonous fishes and ichthyosarcotoxism Armed Forces Med. Jour., 5(2): 157-175, 13 figs., 1 tab.
- Halstead, B. W. & R. J. Ralls
1954 Results of dialyzing some fish poisons. Science, 119: 160-161
- Hashimoto, Y.
1956 A note on the poison of a barracuda, Sphyraena picuda, Bloch and Schneider. Bull. Jap. Soc. Sci. Fish. 21: 1153-1157.

- Hiyama, Y.
1943 Report on the research on poisonous fishes of the South Seas. Nissan Fisheries Experiment Station, Odawara, Japan: 1-137, 29 pls. (In Japanese)
English translation: Poisonous fishes of the South Seas. Spec. Sci. Rept., U. S. Fish and Wildlife Serv., (25): 1-188. 1950.
- Hiyama, Y.
1950 Special scientific report. Fisheries No. 25, United States Dept. of the Interior, Fish and Wildlife Service, Washington, D. C., 1950.
- Jensen, A. S.
1914 The selachians of Greenland. Mindeskript J. Steenstrup. Copenhagen, 30: 12-16.
- Kawabata, T.
1956 An outbreak of squid and octopus poisoning. In litt., March 6, 1956.
- Kawabata, T.,
Ishizaka, K.,
and Miura, T.
1955a Studies on the allergy-like food poisoning associated with putrefaction of marine products II. Separation of causative substance and some of its chemical characteristics. Japanese Jour. Med. Sci. and Biol. 8(6): 503-519.
- Kawabata, T.,
Ishizaka, K.,
and Miura, T.
1955b Studies on the allergy-like food poisoning associated with putrefaction of marine products III. Physiological and pharmacological action of "saurine", a vagusstimulant of unknown structure recently isolated by the authors, and its characteristics in developing allergy-like symptoms. Japanese Jour. of Med. Sci. and Biol., 8(6): 521-528.
- Kerr, W. M.
1912 A note on a case of fish poisoning in Guam. U. S. Nav. Med. Bull., 6: 401-402.
- Legroux, R.,
J. C. Levaditi,
G. Boudin, and
D. Bovet
1946a Intoxications histaminiques collectives consecutives a l'ingestion de thon frais. Presse Med., 29: 545.
- Legroux R.,
J. C. Levaditi,
G. Boudin, and
D. Bovet
1946b Apropos des intoxications histaminiques collectives d'origine alimentaire. Presse Med., 53-743.
- Mann, W. L.
1938 Fish poisoning in Culebra - Virgin Islands area. U. S. Nav. Med. Bull., 36(4): 631-634.

- Neilson, J. L. 1927 Report of an outbreak of fish poisoning on board the U. S. S. "California." U. S. Nav. Med. Bull., 25(2): 480-484.
- O'Neill, J. B. 1938 Food poisoning. U. S. Nav. Med. Bull., 36(4): 629-631.
- Osawa, K., and S. Furukawa 1885 (Physiological effects of puffer poison on laboratory animals.) Report to Tokyo Med. Soc. meeting. Cited by Osawa (1921).
- Ralls, R. J., and B. W. Halstead 1955 Moray eel poisoning and a preliminary report on the action of the toxin. Amer. Jour. Trop. Med. Hyg., 4(1): 136-140.
- Sauvages 1764 De venenatis Galliae animalibus. Thesis, Rothomagensi Academia, 1758. MonsPELLI 1764. P. 6. Cited by Autenrieth (1833).
- Savtschenko, P.M. 1882 A case of poisoning by fish poison. Medits. Pribav. Morsk. Sborniku, St. Petersburg, (9): 55-61. (In Russian).
- Schneck, H. 1945 An unusual clinical syndrome following ingestion of fish. Amer. Jour. Digest Dis., 12(10): 347-350.
- Spear, R. 1904 Fish poisoning. Rept. Surg. Gen. U. S. Navy: 283-284.
- Suehiro, Y. 1947 Practice of fish physiology. Poison of globe fish. Takeuschi Bookstore, Tokyo: 140-159. (In Japanese)
- Tsuda, K., and M. Kawamura 1952 The constituents of the ovaries of globefish. VII. Purification of tetrodotoxin by chromatography. Jour. Pharm. Soc. Japan, 72 (6): 771-773.
- Tsuda, K., and M. Kawamura 1953 The constituents of the ovaries of globefish. VIII. Studies on tetrodotoxin. Pharm. Bull., Tokyo, 1 (2): 112-113.
- Vonfraenkel, P. H., & E. S. Krick 1945 Fish poisoning by barracuda in the Marianas. U. S. Nav. Med. Bull., 44(2): 427-431.
- Walker, F. D. 1922 Fish poisoning in the Virgin Islands. U. S. Nav. Med. Bull., 17(2): 193-202, 7 pls. Also in: Trop. Dis. Bull., London, 21(5): 420. Also in: Envenenamento produzido por peixes nas en las ilhas Virgens. Bol. Ofic. Sanit. Pan-Americana, 3: 185-198. 1924.

- Yokoo, A.
1950 Chemical studies on tetrodotoxin. Rept. III,
Isolation of Spheroidine. Nippon Kagaku Zasshi,
71 (11): 590-592.
- Yokoo, A., &
S. Morosawa
1955 Studies on the toxin of a globefish. Rept. V. Com-
parison with tetrodotoxin. Jour. Pharm. Soc. Japan,
75 (2): 235-236.
- Yudkin, W. H.
1945 The occurrence of a cardio-inhibitor in the ovaries
of the puffer, Spheroides maculatus. Jour. Cell.
Comp. Physiol., 25(2): 85-95, 3 figs.

A P P E N D I X C

**CHEMICAL DETERMINATION OF
PARALYTIC SHELLFISH POISON IN CLAMS**

by

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P U B L I C H E A L T H S E R V I C E

CHEMICAL DETERMINATION OF PARALYTIC SHELLFISH POISON IN CLAMS

The only method in general use for the assay of paralytic shellfish poison is the mouse test developed by Sommer (6) or modifications of this procedure (2). Although the bioassay appears quite satisfactory for the assay of high toxicity shellfish, it may underestimate the poison in those of low toxicity by as much as 60 per cent, due to the attenuating effect of salt (4). It also leaves much to be desired in cases where mice are difficult to obtain and keep in condition for assay. A chemical test for the poison would eliminate these difficulties. Schantz, Mold, and Lynch (5) have reported that the purified poisons from the Alaska Butter clam (Saxidomus giganteus) and the California Ocean Mussel (Mytilus californianus) give characteristic colored compounds with 3, 5-dinitrobenzoic acid or picric acid in alkaline solution. They also presented evidence that these poisons are similar if not identical substances (3) and that the structural part of the poison molecule responsible for the toxicity is also involved in the color reactions.

In a previous paper (4) the authors have reviewed the procedures for the bioassay of paralytic shellfish poison and have suggested the use of the purified poison as a reference standard. The present paper describes studies on the application of one of the chemical tests mentioned above, the reaction with picric acid, to the determination of the poison in shellfish products.

CLAMS STUDIED

Poisonous clams were obtained from the region of Baie des Capucins on the St. Lawrence River and from Ketchikan, Alaska. The Canadian clams were the soft shelled Mya arenaria and were shipped frozen as either whole clams packed in seaweed or as shucked clams in a metal container. The Alaska Butter clams (Saxidomus giganteus) are the species commonly found to be toxic in the Alaskan area. They were received shucked and frozen in sealed metal cans. Nonpoisonous clams Schizothaerus nuttallii, Saxidomus giganteus, and Mya arenaria were received frozen in paper ice-cream-containers from Yaquina Bay, Oregon. Nonpoisonous clams were also obtained from Anchorage, Alaska. The clams in all cases were still partially frozen when received. Upon receipt each batch was thawed, ground in a food chopper and placed in cans for storage at -15°C until needed. Commercially canned clams (probably Artica islandia) were purchased from the local market.

METHOD

REAGENTS

Sodium picrate - Dissolve 1.2 g of reagent grade picric acid (dried at 80-90°C for 1 hour) and dilute to 100 ml with 0.04 N sodium hydroxide.

Sodium hydroxide - Dissolve 3.6 grams of reagent grade sodium hydroxide pellets in distilled water and dilute to 100 ml.

Purified shellfish poison* - Use either crystalline powder or a Stock solution containing 100 ug per ml in 0.001 N hydrochloric acid for preparation of working standards.

Trichloroacetic acid - To prepare 0.5 N solution, dissolve 81.7 grams of reagent-grade trichloroacetic acid in distilled water and adjust volume to 1000 ml.

Buffer solution - Prepare McIlvaine's Standard Buffer Solution of pH 5.2, by mixing 464 ml of 0.1 M citric acid with 536 ml of 0.02 M disodium phosphate.

Ion exchange resin - Amberlite XE-64 cation exchange resin may be obtained from Rohm and Haas Company.

Ether - Reagent grade diethyl ether is washed two to four times with an equal volume of distilled water.

APPARATUS**

Spectrophotometer - Beckman Model B, with 1 cm light path pyrex absorption cells.

Chromatographic tubes - Glass tubes 150 mm long by 19 mm inside diameter, equipped with Ultramax valve, Fisher and Porter Company, Hatboro, Pennsylvania.

Test tubes - Lipless, 150 x 16 mm, medium wall test tubes.

Water baths - A 38°C thermostatically controlled water bath and a boiling water bath, Army Medical School Model, Fisher Scientific Company.

Separatory funnels - 500-ml, pear-shaped, separatory funnels. These funnels are used to feed solutions through the ion exchange column.

*Investigators interested in obtaining small amounts of the solution for use as a reference standard may direct their requests to the Public Health Service, Washington 25, D. C., Attention: Shellfish Sanitation Section.

**The name of the manufacturer is given for some materials and equipment, purely as a matter of identification.

PREPARATION OF RESIN AND COLUMN

About 200 grams Amberlite XE-64 cation exchange resin in the hydrogen form, as received, is slurried with an equal amount of water, allowed to settle for exactly two minutes, and the supernatant immediately decanted to remove the very fine particles which would tend to reduce the flow of liquid through the resin in the column. This operation must be repeated ten times in order to obtain a resin suitable for use. If large amounts of the resin are treated at one time, the material obtained through decantation may be repeatedly subjected to the same operation to obtain additional quantities of resin of the desired particle size. The resin thus obtained is converted to the sodium form by four repeated treatments with 4 per cent sodium hydroxide, thoroughly washed with water, adjusted to pH 7.0 with hydrochloric acid, and washed again with water. A slurry of this material is then poured into the chromatographic tube fitted with a cotton plug in the bottom and the resin is stirred gently to remove intrapped air. The resin is then allowed to settle by gravity and the flow of water through the column until a bed 6.5 cm in height has formed. A cotton plug is placed on the top of the resin and the column is washed with McIlvaine's standard buffer solution (about 200 ml) until the effluent is approximately pH 5.2. Caution must be exercised at all times during the preparation and operation of the column to prevent the entrapment of air in the resin and in or under the cotton plugs. Unless a continuous fluid phase is maintained throughout the column, channeling will result and the column will fail to serve its intended function.

PROCEDURE

Extraction of the poison - A representative sample of the raw shellfish meat or product is ground in a food chopper having 1/8 to 1/4 inch holes in the grinding face. One hundred grams of the freshly ground material is weighed into a beaker and mixed with 100 ml of 0.5 N trichloroacetic acid. This mixture is boiled for 5 minutes and cooled to about room temperature. The pH should be about 1.5 to 2.5. The mixture is adjusted to a volume of 200 ml and filtered by gravity through Whatman No. 12 filter paper.

With canned clams the entire contents of a can of whole or minced clams are placed in a Buchner funnel and drained for 5 minutes. The drained meat is ground and 75 g are weighed into a tared beaker. To each portion of ground meat is added 100 ml of the liquor drained from the product, 25 ml of water, and 6 to 8 g of crystalline trichloroacetic acid (sufficient to obtain a pH of about 2.0). This mixture is then boiled for 5 minutes, cooled, adjusted to 200 ml and filtered.

Adsorption of the poison on an ion-exchange column - One hundred ml of the clam filtrate is adjusted to a pH of 5.0 - 5.2 and passed through the ion exchange column at a rate of 30 drops per minute. After completion of this step, the column is washed with 200 ml of McIlvaine's buffer, followed by 200 ml of water.

Elution of the poison - The poison is removed from the column with 0.5 N trichloroacetic acid, and elution begins as soon as the pH drops below 4.5. By affixing a one-half inch strip of Congo red indicator paper to the tip of the column, the point of emergence of the toxin can be empirically determined. About the first 18 ml represents the void volume and can be discarded. The moment the Congo red paper changes from red to blue the collection of the poison-containing fraction is started. The next 20 ml will contain all of the poison. This fraction is then extracted five times with an equal volume of purified ether to remove the trichloroacetic acid.

Development of color - To 4 ml of the trichloroacetic acid-free eluate is added 1 ml of 1.2 per cent picric acid and 1 ml of 3.6 per cent sodium hydroxide. A blank containing 4 ml of distilled water plus the 1 ml of picric acid and 1 ml of sodium hydroxide is similarly prepared. Both are incubated in a water bath at 38°C for 20 minutes to allow color development, after which the density of the solution is read at 510 mμ, using the blank to zero the instrument. Another 4 ml aliquot of the unknown solution and a blank are similarly prepared and placed in a boiling water bath for exactly fifteen minutes. The density of the unknown, developed at boiling, is likewise read at 510 mμ, using its corresponding blank to zero the instrument.

Calculation of Results - The concentration of poison in the unknown solution is determined by substituting the experimental values in the following equation:

$$P = \frac{150 d_{38} - 28 d_{100}}{0.4}$$

Where P equals the micrograms of poison in the aliquot, d_{38} equals the density of the sample determined at 38°C and d_{100} the density obtained at 100°C. By dividing the ug of poison in the aliquot by 4 and multiplying by the volume of the eluted poison fraction (20 ml) the quantity of poison present per 100 ml added to the column is determined. Since the volume of clam extract was 200 ml, with only 100 ml of filtrate being used, the quantity of shellfish poison present per 100 ml multiplied by 2 gives the ug of poison present per 100 g of meat. In short $P \times 10$ is equal to the number of ug in the sample.

Preparation of Standard Curves - Ten milligrams of purified paralytic shellfish poison (70°C, 25 130 ± 5, water) are weighed and dissolved in 0.001 M hydrochloric acid. Since the poison is hygroscopic, great care should be exercised during weighing. This solution is diluted to the proper concentration with 0.001 N hydrochloric acid and a standard curve is constructed relating the density of the color formed in the Jaffe test to the concentration of poison. The quantities used should be between zero and 200 ug. Straight line relationships are obtained with these quantities when the reaction is carried out at either 38°C for 20 minutes or at 100°C for 15 minutes.

The interfering material is obtained by processing samples of non-poisonous clams in the same manner as is described for the chemical determination of poison in toxic clams. A higher yield of the substance is obtained if aged or slightly decomposed clams are used. One, two, three, and four ml aliquots of the trichloroacetic acid-free eluate from these clams are reacted with alkaline picric acid at 38°C for 20 minutes and at 100°C for 15 minutes. The aliquots of less than 4 ml are adjusted to 4 ml with distilled water.

If the densities obtained on each of these aliquots are plotted on the standard curve for shellfish poison at 38°C, the corresponding amounts of interference in terms of ug of shellfish poison can be determined. For example, if 1 ml of the interfering material developed at 38°C gave a density of 0.02, it is seen from the standard curve for shellfish poison at 38°C (Figure 3) that this corresponds to 7 ug, and 2 ml which gave a density of 0.039, corresponds to 14 ug, etc. The densities of the aliquots developed at 100°C are then plotted against their corresponding concentrations and another straight line results. Together, these standard curves for the interfering material and the poison are used to derive the equation for calculating the amount of poison present.

RESULTS AND DISCUSSION

The preliminary operations of collecting, opening, washing, draining, and grinding the clams for chemical assay are identical with the procedures described previously for the bioassay (4). However, extraction of the shellfish meats in the usual manner with dilute hydrochloric acid was not satisfactory, because a large amount of colloidal material remained in solution and interfered with the subsequent filtration and adsorption on the ion-exchange column. Numerous methods were tried to obtain clear extracts including acidified methanol and ethanol, lead acetate, ferric chloride, charcoal, and celite. Thus far, the most satisfactory method has proved to be the addition of 100 ml of 0.5 N trichloroacetic acid to 100 g of ground clams. After boiling the mixture for 5 minutes, extracts were obtained that could be readily filtered and passed through the ion exchange resin without difficulty.

The ratio of liquid to solids in canned clams is generally about 75 g of meat to 100 ml of bouillon. These proportions have been used in assaying canned clams since they also give a quantity of filtrate about equivalent to that obtained when using 100 grams of raw clam meats. The results, however, have been calculated as if 100 grams of raw clams had been used. Because of the volume of bouillon present, it is necessary to add crystalline trichloroacetic acid rather than 100 ml of 0.5 N solution. Six to 8 g of the acid are required to obtain the desired acidity.

The optimum pH of adsorption of shellfish poison on XE-64 resin is about 5.2. At higher pH values some of the poison is either destroyed by the alkaline conditions or is washed off the column by the buffer. The poison can be adsorbed at a pH as low as 4.5, but as adsorption

progresses the solution may decrease in pH sufficiently to cause partial elution of the poison.

In practice, using the column size herein described, the test is limited to about 6000 mouse units or 1000 ug because the capacity of the resin is exceeded as a result of the adsorption of salts and interfering substances in addition to the poison. If necessary, a larger column can be used, but it is preferable to use the standard size and reduce the amount of filtrate applied to 25 or 50 ml. In this case, of course, the amount of poison found in the filtrate must be multiplied by either 3 or 4 respectively, to determine the amount of poison present per 100 g of meat.

In an effort to reduce the amount of interfering substances in the eluate, a variety of column-washing procedures were investigated. From the results of many experiments it was concluded that 200 ml of McIlvaine's buffer, followed by 200 ml of water, was generally most effective in removing the nonadsorbed impurities and substances that interfere in the color test. The water wash serves primarily to remove the residual buffer salts which interfere in the color tests. Neither different proportions nor greater quantities of the column washes appreciably improve the results.

Although hydrochloric acid may be used to elute the poison from the column, its use presents a considerable problem because the Jaffe test must be performed on a neutral and unbuffered eluate. Most of the hydrochloric acid can be removed by an anion-exchange column or by evaporation to dryness. Since both of these operations are difficult to accomplish, other methods were investigated. Trichloroacetic acid was found to be the most practical elutriant because it is readily removed by ether extraction. The most satisfactory volume in which to collect the poison eluted from the column on which either a low or a high toxicity sample were adsorbed was found to be 20 ml. Although 1 N acid will elute the poison in a slightly smaller volume, an excessive number of ether extractions are required to prepare the eluate for the color test. The sensitivity of the Jaffe test would be increased if all of the poison could be collected in a smaller volume. This could be done, however, only at the risk of decreasing the overall accuracy of the test by failing to collect all of the poison.

Application of the Jaffe test at 38°C for 20 minutes on eluates of toxic clams always yields higher values than the corresponding bioassay of these eluates. These results indicate that a nonpoisonous Jaffe positive substance(s) is adsorbed and eluted from the resin along with the poison. The amount of this substance in toxic clams is represented by the difference between the bioassay on the eluate and the Jaffe test. It averages 87 ± 27 ug per 100 g of meat, as shown in Table 1. Similar studies were conducted to determine the amount of Jaffe positive substances per 100 g of nontoxic clams, and it was found to average 92 ± 16 ug per 100 g. Since these values do not differ significantly from each other, it was concluded that 90 ug represents the average amount of interfering material per 100 g of clams.

The amount of interference is related, however, to the freshness or state of decomposition of the shellfish. With fresh frozen whole clams the above values appear to be reasonably constant, but as the product ages and decomposition occurs, this value may increase considerably. For example, with *Mya arenaria* held for one week at 4°C, the interference increased from 112 to 237 ug per 100 g; and after an additional week of storage at room temperature (badly decomposed), this value rose to 574 ug per 100 g. A portion of the same sample held frozen for three weeks did not change from the original value.

Diethyl ether, used to extract the trichloroacetic acid from the eluate, may contribute significantly (densities 0.25 or greater) to the amount of interfering substances obtained. Even the best grades of ether contain an interfering material. It has been possible to effectively eliminate this source of error by extracting the ether two to four times with an equal volume of distilled water. No other technique has been found to give equivalent results. The "purity" of the washed ether should be checked by extracting five 20 ml aliquots of the ether with the same 20 ml portion of distilled water. Four ml of this water extract and a distilled water blank should then be developed in the Jaffe test at 100°C for 15 minutes. Using the blank to zero the instrument, the density of the extract should not be greater than 0.05 and is generally less.

One of the chief difficulties with the Jaffe reaction is its lack of specificity. In addition to shellfish poison, positive reactions are obtained with a variety of substances including glucose, acetone, creatinine, indole and histamine. When the test is conducted at 100°C, additional substances such as certain alcohols, amines, ketones and aldehydes may also react. The specificity of this test for shellfish poison depends upon selective adsorption and elution of the poison from an ion-exchange resin. Under the conditions of the test, most of the above substances are not adsorbed. However, some substances such as histamine and ethanol (through combination with the poison) may appear in column eluates. Histamine appears to be most nearly like the interfering substance since its adsorption characteristics and reactions in the Jaffe test are practically identical to the interfering material from clams.

Another difficulty with the Jaffe reaction is that the poison complex cannot be read at the point of maximum absorbance (about 480 mμ) because absorbance of the picric acid is considerable at this wavelength. The wavelength of 510 mμ was chosen because the difference between the absorption of light by the picric acid and the picric acid complex of the poison is maximal at this wavelength (Figure 1). This observation is in agreement with the work of Clark, et al. (1) on creatine. Also presented in Figure 1 is the spectral transmittance curve for the picric acid complex of the interfering substance isolated from nonpoisonous clams, which appears to have a wavelength of maximum absorption slightly different from shellfish poison. However, these differences are not sufficient to confirm the separate identity of these substances.

In an attempt to differentiate between the poison and the interfering substances, and to make measurements at the wavelength of maximum absorption possible, means of removing the unreacted picric acid from the reaction mixture were studied. Collidine was the only solvent, among many tried, that would extract the unreacted picric acid from the aqueous solution, leaving behind the developed color. The spectral transmittance curves shown in Figure 2 indicate that the reaction products of the interfering material and the purified poison are quite different.

Although these curves suggest that these two substances might possibly be distinguished on the basis of their transmission curves, it was found that these differences were not great enough to give reliable results when using the Beckman Model B spectrophotometer. The extraction also presents some problems, since the collidine (whose critical solution temperature is near room temperature) may cause the formation of opalescent solutions, making accurate measurements impossible.

The ideal conditions for color development are 38°C for 20 minutes. Between 15 and 25 minutes at this temperature the density changes very slowly, but appreciably lower densities may be observed if shorter or longer times of heating are employed. At 38°C for 20 minutes the density is greater than under any other conditions studied.

In order to differentiate between the amount of interference and poison present, however, it is necessary to develop the color also at 100°C. Fortunately, as shown in Figure 3, when the interfering material is heated in a boiling water bath to develop the maximum color in the Jaffe test, the density is increased about 5-fold over that obtained at 38°C, whereas the density of the shellfish poison at 100°C is about one-third of that at 38°C. The density of the interfering material when heated at 100°C reaches a maximum at 15 minutes, and the density of the shellfish poison decreases to a nearly constant minimum at any time after 5 minutes. In Figure 3 standard curves are given for the color developed when alkaline picric acid is reacted with different concentrations of the poison and of the interfering material at 38°C for 20 minutes, and in boiling water for 15 minutes. These curves were produced by measuring the densities of the solutions in a spectrophotometer at 510 mμ, and plotting the data as described in the methods section.

The conditions of heating must be carefully controlled in order to obtain reproducible results. For this purpose a large metal water bath (8 inches in diameter by 4 inches high) was heated over a bunsen burner. The boiling water was adjusted to the level of the liquid in the tubes which were held upright in a metal rack about one-half inch off the bottom of the bath. Before placing in the bath the test tubes (150 x 16 mm, medium wall) containing the solutions for color development, the temperature should be checked to determine that the water is at 99.5 to 100°C. The solutions must remain in the bath for exactly fifteen minutes, and should be allowed to cool at room

temperature for about 10 minutes before being poured into the spectrophotometer cells (1 cm light path) for reading. At first the density increases slightly as the tubes cool, but the color becomes quite stable on standing and should be measured 10 to 15 minutes after removal from the water bath.

It is entirely unsatisfactory to place the test tubes containing the solutions for color development in a beaker of boiling water with the test tubes resting on the bottom of the water bath, for the heat transfer is such that the density of the color developed may be considerably greater and the results extremely variable.

From the curves in Figure 3 an equation can be derived for calculating the amount of poison in a solution containing an unknown amount of interference. Let

I = ug of interference in the aliquot tested

P = ug of poison in aliquot tested

d_{38} = density of interference plus poison at 38°C for 20 minutes

d_{100} = density of interference plus poison at 100°C for 15 minutes

From the slopes of the curves in Figure 3 it is found that:

Density of interference at 38°C = $.0028 I$

Density of poison at 38°C = $.0028 P$

Therefore,

$$(1) \quad .0028 I + .0028 P = d_{38}$$

Also from Figure 3:

Density of interference at 100°C = $.015 I$

Density of poison at 100°C = $.00086 P$

Therefore,

$$(2) \quad .015 I + .00086 P = d_{100}$$

or

$$(3) \quad I = \frac{d_{100} - .00086 P}{.015}$$

By substituting the value for I obtained in equation 3 into equation 1,

$$(4) \quad .0028 \frac{(d_{100} - .00086 P)}{.015} + .0028 P = d_{38}$$

and solving for P, it is found that,

$$(5) \quad P = \frac{150 d_{38} - 28 d_{100}}{0.4}$$

Since by definition P is equal to the micrograms of poison, this equation can be used to calculate the amount of poison from the density readings obtained on a column eluate when developed at 38°C for 20 minutes and at 100°C for 15 minutes.

To verify this equation various amounts of the interfering material isolated from nonpoisonous clams were added to known amounts of shellfish poison as shown in Table 2. The densities of the solutions were determined at the two temperatures, and the quantity of poison present was calculated by using the above equation. Since the mean deviation of the calculated poison content of these solutions was found to be -1.09 ± 1.23 , it is reasonable to assume that equation 5 is unbiased, namely, that the mean deviation is really zero. Therefore, the standard deviation of the differences between the amount recovered and the amount added can be used as a measure of the precision of this method of assay. As shown in Table 2, the standard deviation is ± 4.56 ug. and the standard error of the mean difference is ± 1.22 ug.

To substantiate further the usefulness of this equation poisonous clams and nonpoisonous clams, to which known amounts of purified poison has been added, were subjected to the bioassay and chemical assay procedures. The equation was then used to calculate the amount of poison present in the column eluates and this value was compared with the results of the bioassay on the filtrate and column eluates.

As seen in Table 3, the bioassays on the column eluates agree well with the chemical assays of these eluates, with the exception of samples A + 100 ug, B and C. Some of the error in sample A + 100 and B is related to the fact that density readings of less than about 0.03 can not be made accurately, and to the fact that, as indicated in Table 2, the precision of the chemical assay is no better than about ± 45.6 ug per 100 g. Actually, the presence of interfering materials, even from non-toxic clams, will give densities of 0.02 or greater, and the presence of as little as 40 ug of poison may be detected since this amount will increase the density to 0.03 or above. However, as indicated in Table 3, because of the combination of the above two sources of error, the lower limit at which measurements can be made by the chemical assay with any degree of accuracy is about 10 to 15 ug per 4 ml or 100 to 150 ug per 100 g of clam meat. Although, this quantity is above the maximum concentration which is presently accepted for human consumption (i.e., 400 mouse units or 72 ug per 100 g), as determined by bioassay, it should be recalled that the bioassay may underestimate the poison in clams of low toxicity by as much as 60 per cent (5). Thus, 72 ug as determined by bioassay of a crude extract may actually represent as much as 180 ug

or about 1000 mouse units. The error observed in the chemical assay of sample A + 100 is, therefore, no greater than may occur in the bioassay of similar samples.

In clam samples of unknown toxicity, such as samples B and C, the chemical assay can be assessed only in terms of the bioassay. Since the accuracy of the bioassay at best is about ± 15 per cent, most of the error in sample C and also part of the error in sample B may arise from this source.

The sensitivity of the chemical assay could probably be improved by using a Beckman Model DU or similar spectrophotometer, which would make possible more accurate measurement of low densities. The cost of such an instrument makes it generally not available to the routine control laboratory. For this reason, it has not been used in these studies.

The recovery of poison from the column (bioassay on filtrate divided by bioassay on eluate multiplied by 100) varied from 140 per cent in the assay of low toxicity clams to 70 per cent with clams of high toxicity (Table 3). Provided no great losses occurred on the column, the high recovery with low toxicity clams was to be expected, since it has been shown that the bioassay tends to underestimate the toxicity in filtrates of low poison content (5). With high toxicity clams, however, and nontoxic clams to which large amounts of purified poison had been added, the amount of poison recovered from the column was consistently low. This phenomenon was related to the capacity of the column. When the column size was increased so that complete adsorption of the poison could be obtained, the recovery of the poison was generally about 100 per cent.

SUMMARY

A colorimetric test for the determination of shellfish poison has been developed. This test depends on the selective adsorption and elution of the poison from a trichloroacetic acid extract of clam meat on an XE-64 ion exchange resin, and upon the application of the Jaffe test to the eluate.

The specificity of the test depends upon the ability of the resin to concentrate the poison and to remove many substances which may interfere in the Jaffe test. Most such substances are removed by the resin treatment, but a few, like histamine, may be adsorbed and eluted with the poison, while others, such as ethanol, may combine with the poison, thus interfering in the test. The amount of interfering material varies with the age or state of decomposition of the shellfish. However, when the eluate is heated at 100°C for 15 minutes to develop the Jaffe test, the color density of the interfering material, as measured at 510 m μ , is about 5 times greater than that obtained at 38°C for 20 minutes, whereas the color density of purified poison decreases about one-third under identical conditions. On the basis of these observations, an equation has been developed for calculating the amount of poison present in a column eluate containing variable amounts of interfering material.

At levels of 100 to 150 ug per 100 g of clam meat or above, the chemical assay has a precision of ± 45.6 ug and gives higher and more accurate values than the bioassay because of the adverse effects of salt in the latter. At lower levels, the chemical assay is no better than the bioassay, due to the insensitivity of the tests to these small amounts.

Since the purified poison has been isolated from mussels and clams by similar methods, the colorimetric test is probably applicable to these and several other varieties of shellfish, but toxic samples of species other than clams have not been studied.

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REFERENCES

1. Clark, L. E. and Thompson, H. L. Determination of Creatine and Creatinine in Urine, *Anal. Chem.* 21, 1218 (1949).
2. Medcof, J. C.; Leim, A. H.; Needler, A. B.; Needler, A. W. H.; Gibbard, J. Paralytic Shellfish Poisoning on Canadian Atlantic Coast, *Bull. Fish. Research Board, Canada* 75 (1947).
3. Schantz, E. J., *et al.* A Procedure for the Isolation and Purification of the Poison from Toxic Clams and Mussel Tissue, *J. Am. Chem. Soc.*, 79 5230 (1957).
4. Schantz, E. J.; McFarren, E. F.; Schafer, M. L.; and Lewis, K. H. Purified Shellfish Poison for Bioassay Standardization. (This Journal).
5. Schantz, E. J.; Mold, J. D.; and Lynch, J. Color Tests Given by Shellfish Poison, Chemical Corps, Unpublished Data.
6. Sommer, H. and Meyer, K. F. Paralytic Shellfish Poisoning, *Arch. Path.* 24, 560 (1937).

Table 1 - Assay of Poisonous Clams^a

	Bioassay on Filtrate	Bioassay on Eluate	Color Test on Eluate at 30°C	Difference Between Color Test and Bioassay of Eluate
<u><i>Mya arenaria</i></u>	187	213	297	84
Standard deviation		± 15.6	± 4.2	± 14.9
Per cent deviation		7	2	18
<u><i>Saxidomus giganteus</i></u>	147	172	263	91
Standard deviation		± 17.3	± 36.9	± 26.4
Per cent deviation		10	14	20
<u><i>Saxidomus</i> siphons</u>	298	367	453	86
Standard deviation		± 84.0	± 56.0	± 43.8
Per cent deviation		23	12	50
Average amount of interference				87.3
Standard deviation				± 26.7
Per cent deviation				32

^a All data expressed as ug per 100 g of clam meat.

Table 2 - Recovery of Paralytic Shellfish Poison
Added to Interfering Material Isolated from Nontoxic Clams

Clam Sample	Interference (ml of) (Eluate)	Poison (ug) (added)	Poison (ug*) (Recovered)	Difference (ug recovered) (minus ug added)
A	1	0.5	3.5	+3.5
	1	25	28.0	+2.0
	1	50	48.0	-2.0
	1	100	92.0	-8.0
	2	0	1.8	+1.8
	2	25	24.6	-0.4
	2	50	46.5	-3.5
	2	100	93.0	-7.0
	3	0	8.0	+8.0
	3	25	26.0	+2.0
B	2	0	1.4	+1.4
	2	25	23.4	-1.6
	2	50	45.5	-4.5
	2	100	93.0	-7.0

$$\text{Standard Deviation} = \sqrt{\frac{290.67}{14}} = \pm 4.56$$

$$\text{Standard Error of Mean} = \frac{4.56}{\sqrt{14}} = \pm 1.22$$

* Calculated by use of equation No 5 in text.

Table 3 - Comparison of Bioassay with Chemical Assay
of Shellfish Poison in Clams

Sample	Bioassay on Fil- trate ug per 100g	Bioassay on Elu- ate ug per 100g	Percent Recov- ery	Chemical Assay ug per 100g	Per cent Error ^b	ug of Inter- ference per 100 g
A	0	0	---	0	---	150
A + 100 ug ^a	71	108	108 ^c	160	+39	130
A + 150 ug	75.4	145	96 ^c	138	+7.8	143
A + 300 ug	164	334	111 ^c	350	+4.7	90
A + 300 ug	246	308	103 ^c	284	-7.8	92
A + 400 ug	273	421	105 ^c	386	-9.1	87
A + 500 ug	314	526	105 ^c	538	+2.3	88
A + 600 ug	492	624	104 ^c	592	-5.4	100
A + 800 ug	744	789	97.5 ^c	788	-1.3	81
B		83	92 ^d	100	+20	275
B + 166 ug	192	277	140 ^d	250	-9.6	361
B + 232 ug	300	395	130 ^d	394	-0.3	367
C	137	137	100 ^d	155	+13	363
D	1480 _d	1040	70 ^{d,e}	965	-7.2	420

^a ug of purified poison added per 100 g of meat.

^b $\frac{\text{Chemical assay} - \text{eluate bioassay}}{\text{eluate bioassay}} \times 100$

^c $\frac{\text{Bioassay on Eluate}}{\text{ug of poison added}} \times 100$

^d $\frac{\text{Bioassay on Eluate}}{\text{Bioassay on Filtrate}} \times 100$

^e Capacity of column exceeded

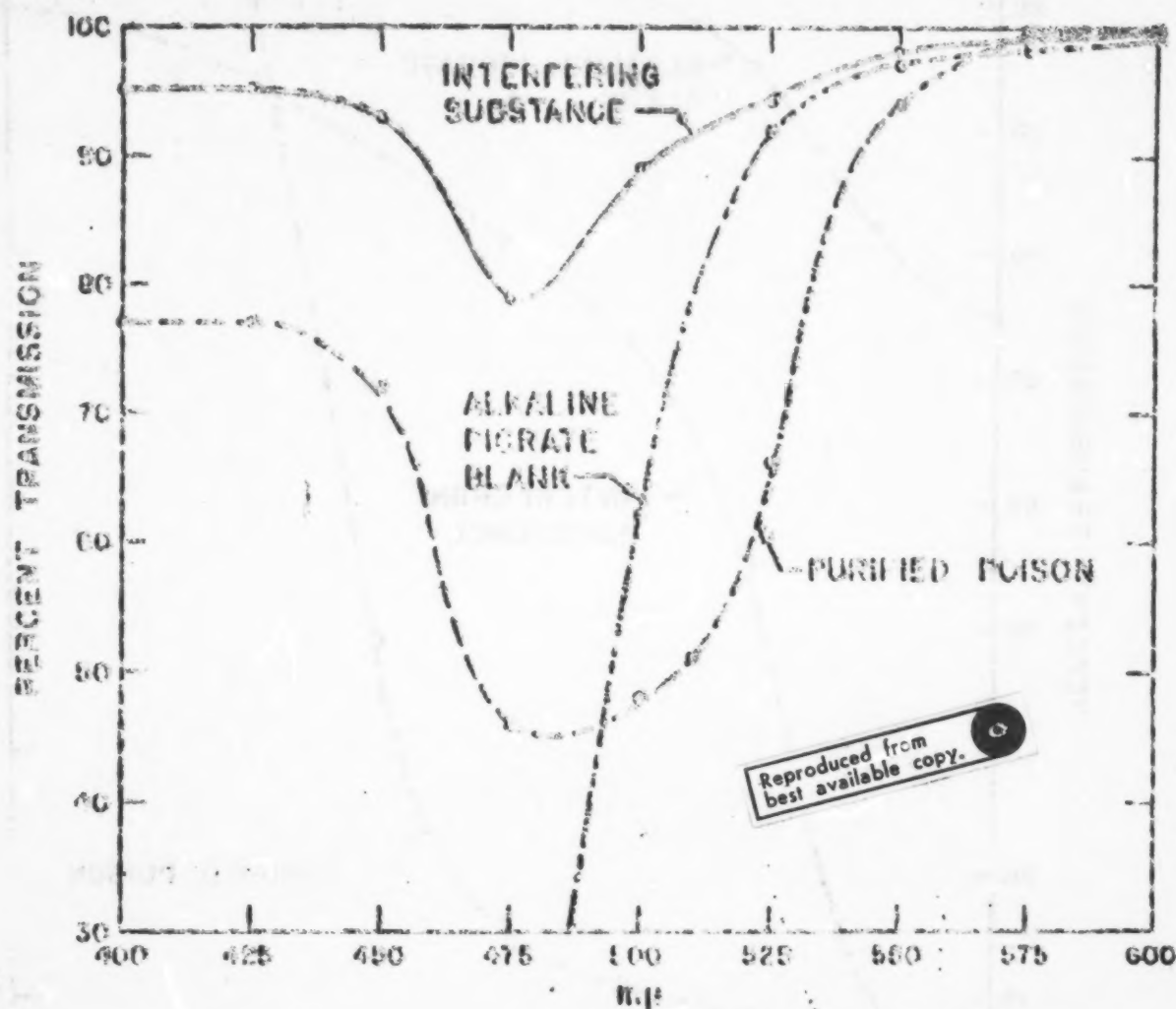


FIGURE 1. SPECTRAL TRANSMISSION OF REACTION PRODUCTS OF THE JAFFE TEST

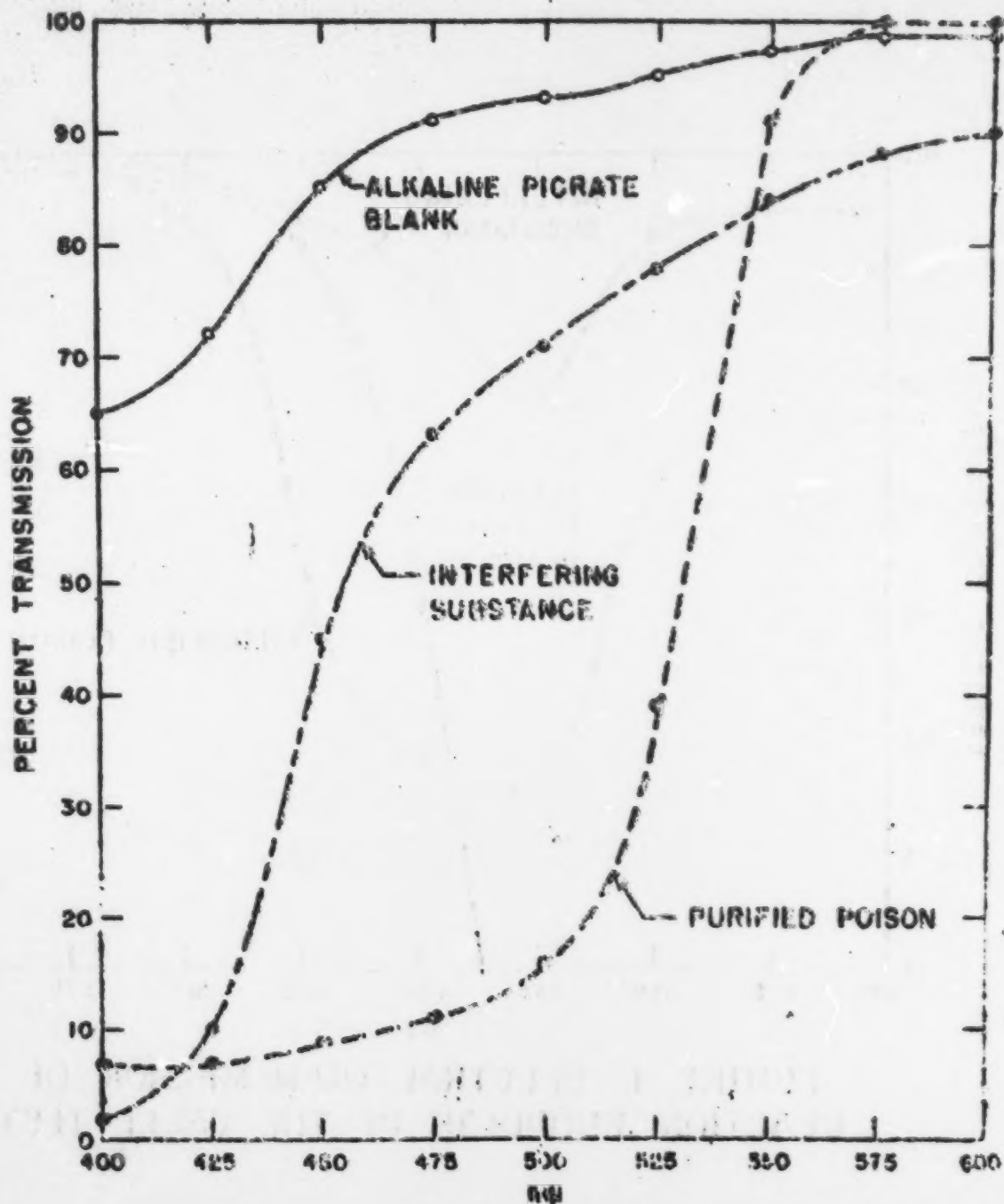


FIGURE 2. SPECTRAL TRANSMISSION OF COLLIDINE EXTRACTED REACTION PRODUCTS OF THE JAFFE TEST

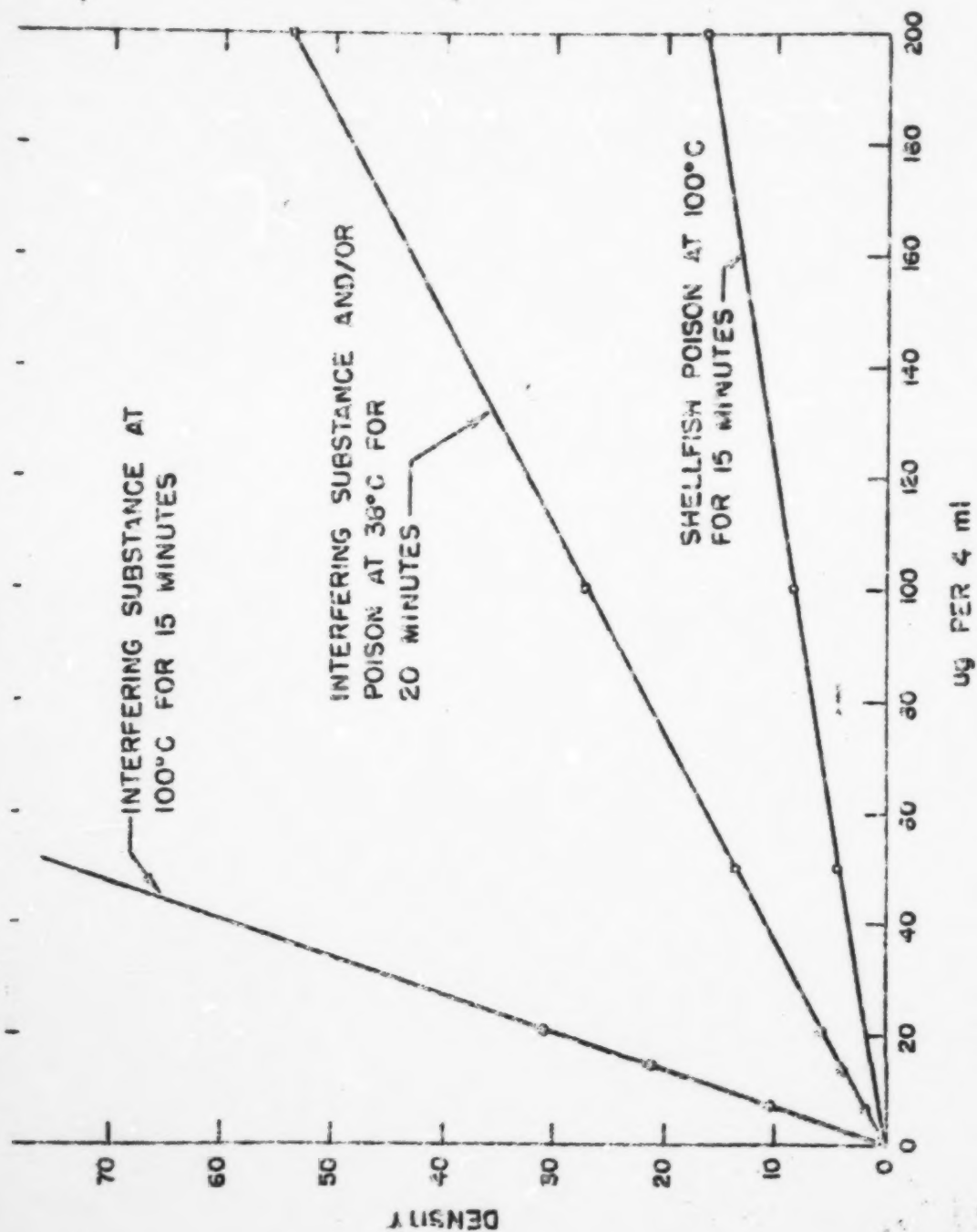


FIGURE 3. STANDARD CURVES OF SHELLFISH POISON AND INTERFERING SUBSTANCE IN THE JAFFE TEST

A P P E N D I X D

EPIDEMIC SHELLFISH POISONING
IN NEW BRUNSWICK, 1957

by

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DEPARTMENT OF FISHERIES
CANADA

INTRODUCTION

On June 24, 1957, more than twelve years after the last certainly established case of paralytic shellfish poisoning in the Fundy region, a telephone call from a news reporter of "The Telegraph Journal", Saint John, N. B., provided us with the first information that another outbreak had occurred. Cases reported then and subsequently (total of 32) were all from the New Brunswick side of the Bay of Fundy and resulted from consumption of soft-shell clams (*Mya arenaria*). None were fatal.

ACKNOWLEDGEMENT

The authors are grateful for assistance given them in their study of this epidemic. The Department of National Health and Welfare, particularly the Laboratory of Hygiene, carried out assays of all extracts of shellfish samples taken and kept us informed of the results. Officers of several services of the Department of Fisheries helped in many ways. Locally, Dr. F. Hazen, Medical Health Officer for St. John and Charlotte Counties, kept us constantly informed of developments as they came to his attention. Dr. C. Trask, Director, General Hospital, Saint John, supplied much information and arranged interviews for us with patients who were treated in his institution. Mr. Ralph Costello, Managing Editor of "The Telegraph Journal" and the associated newspaper "The Evening Times-Globe" and his staff of reporters rendered the whole district an important service by keeping them informed of the danger of poisoning and, in so doing, probably prevented much suffering.

EXTENT OF THE EPIDEMIC

Immediately following the telephone communication from the newspaper reporter, the District Medical Health Officer was contacted and he supplied the names of eight cases and the location of the beds from which the clams were supposedly taken. Within four hours of the first report the authors were sampling these clam stocks. Subsequent investigation revealed that the focus of poisoning was at Lepreau Basin, some ten miles distant by water from the areas sampled. Lepreau Basin is sampled weekly at this season as called for in our established management scheme and a sample was taken there on June 24. Thus the clam stocks chiefly concerned in the week-end epidemic were all tested within twenty-four hours of the time the poisonings occurred.

The first case occurred late on the night of June 22, but most of those poisoned dug and ate clams on June 23. As already indicated, none of these cases was reported to us until June 24. Four persons were poisoned on June 30, one on July 3 and one on July 17, bringing the total number of cases to thirty-two. Of these, one resulted from clams that reached the consumer through regular commercial channels. The other thirty-one sufferers ate clams which they dug themselves while on seashore picnics or which were given to them by friends

returning from picnics. Four of these cases were hospitalized, a dozen received medical attention in their homes and the others recovered without medical assistance.

Besides the poisoning of humans we have a record of two cats being fatally poisoned at Maces Bay on July 1, from eating clams. The symptoms were essentially the same as those described by Medcof et al (1947).

ADEQUACY OF MANAGEMENT SCHEME

The most important shellfish areas are regularly sampled as part of the federal government's management scheme and on the basis of samples taken on June 17, the Department of National Health and Welfare recommended closure of Lepreau Basin, Lepreau Harbour and Pocologan clam areas. At no time did the Nova Scotia areas show toxicity and there were no closures there. The quarantined New Brunswick areas were posted with prominent warning notices on the morning of June 22. In spite of these notices, it was only a matter of hours before picnickers began digging the first clams which caused poisoning.

Using the information we gave the reporter who telephoned us, "The Evening Times-Globe" carried a front-page article in its June 24 edition and "The Telegraph Journal" followed with a similar article on June 25. This was immediately copied or paraphrased by several other papers, dailies and weeklies, published in the central and southern parts of the province. They gave a good account of the nature and origin of paralytic poison, acknowledged the source of the information, described the poisonings which had occurred up to then and stressed the realness of the danger of eating clams from Charlotte and St. John Counties, both then and for an indefinitely continuing period. This, they pointed out, would be determined by the Departments of National Health and Welfare and Fisheries. It is hard to imagine how a warning could be made more effective. Three additional feature articles appeared in "The Telegraph Journal" on June 28, July 11 and August 1. The script for one of these was supplied by the Information Service of the Department of Fisheries. In spite of these news releases, residents of shore communities continued to dig and eat clams and this resulted, as already pointed out, in five more poisonings. The newspaper releases did, however, discourage picnickers, for no more poisonings occurred among them. Our efforts to co-operate with the press seem to have been rewarding.

In our last twelve years' efforts to limit the risks of poisoning we have been able to predict the onset of the hazard far enough in advance to post the dangerous clam areas. This year and in 1945, however, there was a very short interval between the time we had evidence of an impending rise in toxicity and the time dangerous scores were actually attained. This has discouraged confidence in our management scheme. Some hold

that a completely new system of control will have to be devised. Some, on the other hand, considering the effectiveness of the present scheme over the last twelve years, believe that it can continue to provide the needed protection through revision in the method of its application.

It is important to have the best possible control in the next few years because we expect that the poison problem will continue to be critical (Bond 1956). This is a matter which must be examined carefully before the onset of the danger season of 1958.

One thing is clear above all others -- no matter how good the control scheme may be and no matter how well it is applied, it will not be effective unless the public is made better aware of the danger. They must be taught the realness of the danger and taught to heed warning notices because these periodic hazards will continue to occur. Any new management scheme or any modification of the present scheme for better protection, should comprehend effort toward public education. The newspapers would be helpful in this as already shown but they may not, by themselves, be sufficient.

This public education problem is not simple. Residents of shore communities generally exhibit a high degree of resistance to shellfish poison and many of them refuse to believe that any danger exists. They appreciate no need for warning picnickers and, indeed, they often encourage them to ignore "government" warning signs and effectively cancel out our best efforts. It is significant, however, that five of the six cases of poisoning which occurred after the main outbreak were among the shore residents themselves. These five and those acquainted with their misfortune, should be susceptible to education from now on.

Samples taken from one shucking plant scored 6,500 mu. (mouse units per 100g. of raw meat) at a time when the highest-scoring Class I areas were showing a maximum of 960. In the danger period commercial digging is legal only in Class I areas. It must be concluded from this that industry violated the regulation which "closed" the dangerous areas. To this extent the present system of control may be regarded as deficient. It is questionable whether industry can be trusted to abide by such regulations in future. This, and the fact that this year Class I areas were mildly toxic (see below) suggests that any revision of the present control scheme should provide for complete closure of all clam areas in the Fundy region of New Brunswick during the danger period. Possibly Nova Scotia should be closed as well. In effect this would mean that Class I areas should henceforth be treated as Class II in our management scheme.

TOXICITY LEVELS IN VARIOUSLY CLASSIFIED AREAS

The early 1957 rise in toxicity proved that June is not a "safe" month. The rise in toxicity in 1949 came on June 28, but that year the scores of clams did not rise very high and there were no poisonings. The early rise was not the only unusual feature of the 1957 outbreak. During the previous fourteen years for which records are available, clams in what have been termed Class I areas have almost never been toxic and when they have, their scores have never reached 400. Furthermore, since 1950, clams in Class II areas, ordinarily toxic during the summer months, have shown no dangerous scores and clams in Class III areas have been affected only seasonally rather than year-round as in the several years preceding 1950. In 1957, however, clams in some Class I areas attained a maximum score of 960 and exceeded the 400 quarantine level for nearly two weeks. Clams in Class II areas achieved a maximum of 2,370 and exceeded the quarantine level for about five weeks. Clams in Class III areas reached a maximum of 17,800 in June and some still retain poison four months after the peak scores were attained.

INVESTIGATION OF CASES OF POISONING

Within seventy-two hours of the first report, the authors had personally interviewed sixteen persons who had been poisoned and through them obtained reliable information on sixteen others, (family members and friends), who had eaten clams on June 22 or 23. This time-consuming work was greatly facilitated by the co-operation of Dr. Trask who permitted access to patients then at the Saint John General Hospital. Dr. Hazen was equally helpful. Data on nine other cases were obtained from answers to questionnaires mailed to them (Table VI). A similar form was completed for each of the cases interviewed. These records comprise Appendix I to this report. The same form was used to compile records of those who ate toxic clams without ill effects. These records are summarized in Table IV. Information on cases which developed after June 23 was assembled as soon as interviews could be arranged.

Our records, (Tables II, III and IV), show that seventy-two persons ate highly toxic clams; that of these, thirty-two exhibited symptoms typical of paralytic shellfish poisoning and that forty were unaffected. Four cases were hospitalized. A dozen others received medical treatment in their homes. The others "sat it out" without medical advice.

ESTIMATED POISON DOSAGES WHICH CAUSED VARIOUS SYMPTOMS AND ILLNESS

In addition to providing information on source, time of digging, method of cooking, etc., each person interviewed, estimated as accurately as possible the number of clams he had eaten (Table VI). Where pertinent results of assays on raw clams were available, the data were used in combination with others to calculate the amount of poison ingested.

At Lepreau Basin the toxicity scored 508 on June 17; 17,800 on June 24 and 11,600 on June 28. The fact that the five cases resulting from clams dug on June 22 were all of the mildest type indicated that the toxicity had not reached its maximum on that date. Since the score on June 28 was down again, it is assumed that the scores were at or near their peak on June 23 and that the 17,800 score of the sample taken June 24, less than twenty-four hours after the poisoning occurred, approximated that of the offending clams.

The estimated dosage for Case No. 2, Table II, is the most accurate on record. When this patient became ill from eating some of the clams she helped gather at Dipper Harbour on June 23, those that were left were discarded. On June 25 the authors were able to recover enough of these live clams from a garbage can to prepare a duplicate set of extracts. These scored 10,200 and 9,700 (Av. 9,850). Since Madcof et al (1947) demonstrated that the toxicity of clams held in air changes very slowly and since the extracts were prepared less than forty-eight hours after digging, it is assumed that these scores are representative of the clams actually eaten by the patient. Size and weight measurements were made of these Dipper Harbour clams when the extracts were prepared. This allowed for an accurate calculation of the weight of meat eaten by the patient. So far as the authors are aware, this is the only such case on record.

Preparatory to estimating dosages for the Lepreau Basin cases of poisoning it was necessary to determine the sizes of clams available there, and the raw weights and the cooked weights of their meats. Accordingly, a half-barrel lot was obtained, the data for which are presented in Table I. The average length of the clam was $2\frac{1}{2}$ inches and data for this size clam were used in all calculations of dosages of poison taken by consumers of clams from this area.

Madcof et al (1947) showed that, on the average, 100g. of cooked meats contain only 30% of the poison contained in 100g. of raw meat. Dosages for those who ate cooked clams were, therefore, calculated as 30% of the raw toxicity per gram multiplied by the calculated weight of cooked meat ingested. The dosage resulting from eating raw clams was similarly calculated omitting the correction for cooking loss.

In 1957, as in 1945, most of the sufferers were picnickers from the city who took advantage of the first hot week-end to flock to the beaches. The clinical picture of those affected was very similar to that of the 1945 outbreak, but differences in effects seem to have depended more on individual differences in susceptibility to poisoning rather than on differences in the amount of poison ingested. Some persons were extremely sensitive; e.g., the two-year-old child (Case No. 6, Table II) who showed advanced symptoms of poisoning from ingesting only 600 mouse units, and the young man (Case No. 15, Table III) who immediately noticed a pins-and-needles feeling around his lips merely from chewing the stomach portion of one raw clam which, incidentally, he afterwards swallowed -- estimated dosage being 1,100 mouse units.

This last case is of particular interest because it indicates that the poison need not be digested to have its effects. His immediate symptoms must have been produced by absorption of the poison through the mucosa of the mouth. This probably explains why numbness of the lips, tongue and throat appears in almost every case of poisoning and appears before any of the other symptoms.

Case No. 1, Table II, is interesting because although only 5,500 mouse units of poison were ingested it is the most severe poisoning ever reported to us excepting, of course, those involving deaths. This patient exhibited the peculiar symptom of loosening of the teeth - three of which came out. The accurate estimate of another 5,500 mouse-unit dosage (Case No. 2, Table II) definitely proves that susceptible persons may suffer severely from a rather low intake of poison.

In contrast, some people have tolerance for great amounts; e.g., Case No. 38, Table IV, felt no ill effects after ingesting 13,400 mouse units and Case No. 16, Table III, experienced only mild symptoms from 25,800.

Medcof et al (1947) obtained some evidence that tolerance to poison may be acquired by residents of shore communities by regularly eating low-scoring clams. This year's records shed no further evidence on this interesting point. They merely show that there are differences in tolerance among both residents and non-residents of shore communities.

The importance of great individual variation in susceptibility has not been sufficiently emphasized in the literature to date. Investigators have been at pains to calculate average dosages required to produce symptoms of different severities (Sommer - See Medcof 1953), (Medcof et al 1947). From Table II it is clear that such averages can hold only academic interest in the minds of those responsible for preserving public health. They must concern themselves not merely with protecting the average person but also those who are more susceptible to the poison than average. Furthermore, in estimating the risk of poisoning it must be remembered that susceptible persons may eat large, not average-sized, meals of shellfish and may eat them raw.

With these points in mind it is pertinent to review what data are available on minimum mouse-unit dosages which caused illness in our region. Tables II and III show that it was 600 in 1957 for a very small child and 1,100 for an adult. The toxicity score of the clams eaten was 17,800. Tennant et al. (1955) recorded 650 for an adult who ate clams scoring approximately 5,500 and Medcof et al (1947) reported 1,000 plus for an adult eating clams scoring 16,000.

CRITIQUE OF 400 UNIT QUARANTINE

It is remarkable that all poisonings have resulted from consumption of high-scoring shellfish. The least toxic clams that induced poisoning in 1957 scored 9,850 (Case No. 23 and 24, Table III); in 1954, 5,500 (Tennant et al 1955) and in 1945, 6,200 (Medcof et al 1947). From what we know now it is theoretically possible to suffer poisoning from low-scoring clams but this has never been observed. Therefore, as concluded earlier (Medcof et al 1947) there would seem to be no reason for abandoning this 400 unit level. For some time now it has been generally accepted as a safe upper tolerable limit for toxicity for commercial packs of clams. Areas have been closed when toxicity exceeded the 400 unit level and not reopened until it fell to a level consistently below 400. There would seem to be no risk in continuing this practice if it is otherwise acceptable.

COMPARISON OF 1945 AND 1957 EPIDEMICS

The 1945 epidemic of poisonings has been reported by Medcof and Gibbons (1945) and Medcof et al (1947) and it is instructive to make comparisons (Table VII) between it and the 1957 outbreak.

The similarities are striking in spite of the fact that there was a twelve year interval between outbreaks. They were about equal in severity judging from the number of cases of poisoning involved. Had it not been for the good newspaper warning and careful posting of dangerous areas, however, the 1957 outbreak might have been much the worse. The focus of poisonings in both outbreaks was Lepreau Basin and the score of the offending clams was the same within 200 units. The minimum scores of clams which caused poisoning were high in both epidemics. The relative levels of the toxicity scores in Class I, II and III areas was roughly the same. In other words the worst areas are always worst and the best are always best. The causative organism was apparently the same. The dinoflagellate Gonyaulax tamaris was taken in plankton tows made by the staff of the Biological Station, St. Andrews, at the time of both outbreaks. In both instances its increase in abundance, which is responsible for the rise in toxicity of the clams, succeeded a sudden rise in water temperatures in the shallow inlets of the Bay of Fundy. This in turn is attributable to a spell of unusually warm, bright weather.

In both outbreaks our system of predicting the toxicity rises in clams failed. They occurred almost simultaneously (not ten days later as in normal years) with the rise in score of Head Harbour mussels.

The outbreaks differed in that both New Brunswick and Nova Scotia sides of the Bay of Fundy were affected in 1945 whereas only New Brunswick areas were involved in 1957. The reason for this is obscure.

Obviously there are many gaps in our understanding of the problems of paralytic poisoning that could be clarified by investigations of various sorts. Our present method of management could be made more effective if we had a quicker method of assay. The greatest need, however, is to discover a more reliable method of predicting danger periods. For this it would seem necessary to attack the problem at its roots by investigating the biology of the causative organism.

SUMMARY

1. In late June and early July 1957, there were thirty-two cases of paralytic shellfish poisoning in southeastern New Brunswick. There were four hospitalizations but no fatalities. For this district this is the largest and earliest-appearing outbreak on record.

2. The soft-shell clam (*Mya arenaria*) was the only species of shellfish involved and all were from the New Brunswick side of the Bay of Fundy. Nova Scotia clams were not affected.

3. The most severely poisoned person exhibited a symptom which has not been previously reported - a temporary loosening of the teeth. In this case three teeth actually came out before the patient recovered.

4. It would seem that shellfish poison may be absorbed directly through the oral mucosa. This would explain why numbness of the tongue, lips and cheeks is the most consistently appearing and usually the first symptom of poisoning.

5. A discarded part of a lot of clams which caused one of the severest poisonings was recovered and assayed. This permitted what is probably our most reliable calculation of poison dosage - 5,500 mouse units.

6. Calculations of poison intake by sufferers and non-sufferers for whom records are available show enormous person-to-person differences in susceptibility - a child became severely ill from a dose of only 600 mouse units while one adult ingested 13,400 units and showed no symptoms whatever.

7. Because of this wide range in susceptibility, calculations of average dosages responsible for illnesses are of little use in framing public health regulations. Those who are more susceptible to the poison than average require protection.

8. One poisoning resulted from clams marketed through regular commercial channels. Apparently there had been illicit digging in quarantined areas in spite of efforts by the Department of Fisheries to prevent this.

9. All other poisonings resulted from consumption of clams dug by picnickers who disregarded prominent warning signs posted at the beaches.

10. Many residents of shore communities have a high tolerance for shellfish poison and hence do not appreciate the risk. They sometimes encouraged visitors to disregard warning signs.

11. The newspaper publicity this year seems to have had desirable effects. Future management schemes should make the fullest possible use of the press both for warning and for education of the public.

12. Normally, the toxicity of mussels at the Key Station rises a week or ten days before clams in the producing areas become toxic. In 1957 and in 1945, when this warning was most needed, the rises were almost simultaneous. This has discouraged confidence in our system of control.

13. The poison problem is expected to be more acute in the next few years than it has been in the past ten. This means that we must devise a better management scheme. A search for improvement should be begun now.

14. A quicker method of assay would make our control method more effective, but what is most needed is a method of long-range prediction of danger periods. The first step in this direction would be careful study of the causative organism.

LITERATURE REFERRED TO

- Bond, R. M., Toxicity Records, 1956. Department of Fisheries, Fish Inspection Laboratory, MS Report 1956.
- Medcof, J. C. and R. J. Gibbons 1945. Paralytic Shellfish Poisoning in Nova Scotia and New Brunswick. Fisheries Research Board Can., MS. Report Biological Stns. 376.
- Medcof, J. C., A. H. Leim, Alfreda B. Needler, A. W. H. Needler, J. Gibbard and J. Naubert 1947. Paralytic Shellfish Poisoning on the Canadian Atlantic Coast. Fish. Res. Bd. Can. Bull. 75: 1-32.
- Medcof, J. C., 1953. Criteria to be Applied to Shellfish Export From Areas Sometimes Affected by Paralytic Shellfish Poison. Appendix II to minutes 1953 meeting of Inter-departmental Shellfish Committee, Ottawa.
- Tennant, A. D., J. Naubert and H. E. Corbeil 1955. An Outbreak of Paralytic Shellfish Poisoning. J. Can. Med. Assoc. 72: 436-439

Table 1. Size composition of a representative half-barrel

sample of clams harvested at Lepreau Basin July 18,

1957, and yields of meat, raw and cooked, from various-

sized clams.

Shell Length (inches)	No. of Clams	Frequency %	Raw meat yield/clam (gm.)	Cooked meat yield/clam (gm.)
2 - 2 $\frac{1}{4}$	345	29	6.3	3.8
2 $\frac{1}{4}$ - 2-3/4	645	57	12.1	5.7
2-3/4 - 3 $\frac{1}{4}$	205	17	21.3	11.8

Table 11. Summary of data from Appendix I on 13 cases of serious shellfish poisoning in New Brunswick in 1957

Case No.	Sex	Age (approx.)	Symptoms												Interval between eating and onset of illness (hours)	Duration of effects (hours)	Number of shellfish eaten (approx.)	Preparation	Date fish- ed 1957	Toxi- city (raw) p/r 100g.	Esti- mated dos- age (m.u.)
			Num- bness of face	Num- bness of lips and tongue	Num- bness of fingers and toes	Nausea	Vomit- ing	Abdomi- nal cramps	Diarrhoea	Headache	Dizziness or giddiness	Num- bness of arms and legs	Prostration	Backache	Diffi- culty in respiration						
1	F	35	X	X	X	X			X	X	X	X	X	X	X	1	180	18	6/23	17,800	5,500
2	F	60	X	X	X		X			X	X	X	X	X	X	1/2	72	21	6/23	9,850	5,500
3	F	28	X	X	X		X				X	X	X	X	X	1/2	60	6	6/23	17,800	7,400
4	F	30	X	X	X				X	X	X	X	X	X		1/2	96	40	6/23	17,800	12,200
5	M	45	X	X	X			X	X	X	X	X	X	X		4	84	30	6/23	17,800	9,100
6	F	2					X				X	X	X	X		< 1	24	2	6/23	17,800	600
7	M	65	X	X	X		X				X	X	X	X		< 1/2	72	20	6/30	---	---
8	M	63	X	X	X						X	X	X	X	X	1/2	48	12	6/23	17,800	3,600
9	F	50	X	X	X			X		X	X	X	X			2 1/2	72	21	6/23	17,800	6,400
10	M	65	X	X	X		X			X	X	X				1/2	72	24	6/23	17,800	51,700
11	M	60	X	X	X					X	X	X				3	36	24	6/23	17,800	29,500
12	M	30	X	X	X					X	X	X		X	X	1/2	96		6/23	17,800	10,800
13	M		X	X								X	X			-	-		6/23	17,800	--

Table III. Summary of data from Appendix I on 19 cases of mild shellfish poisoning in New Brunswick, 1957

Case No.	Sex	Age (approx.)	Symptoms										Interval between eating & onset of illness (hours)	Duration of effects (hours)	Number of shellfish eaten (approx.)	Preparation	Date fish eaten 1957	Toxicity (raw) per 100g.	Estimated dosage (m.u.)			
			Numbness of face	Lips and tongue	Numbness of fingers & toes	Nausea	Vomiting	Abdominal cramps	Diarrhoea	Headache	Dizziness or giddiness	Numbness of arms and legs								Prostration	Backache	Difficulty in respiration
14	F	-	x	x	x	x								12	12	12	raw	6/23	17,800	25,800		
15	M	20	x	x	x	x								18	18	12	"	"	"	17,800	1,100	
16	M	-	x	x	x	x								12	12	12	"	"	"	17,800	25,800	
17	F	55	x	x	x	x								18	18	22	raw &	"	"	"	17,800	25,800
18	F	20	x	x	x	x								48	35	35	stm.	6/22	"	"	17,800	25,200
19	F	25	x	x	x	x								24	36	24	"	"	"	"	"	"
20	F	31	x	x	x	x								24	24	24	"	"	"	"	"	"
21	M	65	x	x	x	x								24	36	36	"	"	"	"	"	"
22	M	26	x	x	x	x								30	36	12	"	"	"	"	"	"
23	M	50	x	x	x	x								6	6	6	"	6/23	9,850	1,900		
24	M	60	x	x	x	x								12	15	15	"	6/23	9,850	2,400		
25	M	8	x	x	x	x								12	16	16	"	"	"	"	"	"
26	M	-	x	x	x	x								<7	24	24	"	"	"	"	"	"
27	M	-	x	x	x	x								<10	15	15	"	"	"	"	"	"
28	F	65	x	x	x	x								12	12	-	"	6/30	---	---	---	---
29	M	40	x	x	x	x								>12	12	-	"	6/30	---	---	---	---
30	F	38	x	x	x	x								48	9	9	"	"	"	---	---	---
31	M	-	x	x	x	x								-	-	-	-	7/17	---	---	---	---
32	F	21	x	x	x	x								>4	12	-	fried	7/3	---	---	---	---

Table IV Records of 1957 consumptions of toxic New Brunswick clams without harmful effects.

Case No.	Description	Age (approx.)	Cross Ref. Case No.	Number clams per person (approx)	Preparation of clams	Date fished	Source	Toxicity per 100g. mouse units	Estimated dosage per person (m.u.)
<u>Non-residents of shore communities</u>									
33	Man	50	9	6	steamed	6/23	Lebanon Basin	17,800	1,800
34	Boys (3)	7-14	9	12	"	"	"	17,800	3,600
35	Man	30	4	24	"	"	"	17,800	7,300
36	Boys (2)	7 & 4	4	4	"	"	"	17,800	1,200
37	Man	40	1	30	"	"	"	17,800	9,100
38	Man	55	12	32	raw & steamed	"	"	17,800	13,400
39	Group (16)	Adults & Children	12	12	steamed	"	"	17,800	3,600
40	Woman	60	10	6	raw	"	"	17,800	12,900
41	Man	31	18	24	steamed	6/22	"	-	-
44	Man	35	18	35	"	"	"	-	-
45	Boy	10	10	-	"	"	"	-	-
46	Woman	-	18	35	"	"	"	-	-
47	Man	-	18	35	"	"	"	-	-
48	Woman	30	3	-	raw & cooked	6/23	"	-	-
49	Man	22	32	60¢ dish	Fried	7/3	Ganteen, St. John	-	-

*These numbers refer to cases listed in Tables II and III who were poisoned by sharing the same lots of clams as these non-sufferers.

(Con't.) Table IV Records of 1957 consumptions of toxic New Brunswick clams without harmful effects.

Case No.	Description	Age (approx.)	Cross Ref. Case No.	Number of Clams per person (approx.)	Preparation of clams	Date fished	Source	Toxicity (raw) per 100g. mouse units	Estimated dosage per person (m.u.)
<u>Residents of shore communities</u>									
42	Boy	13	7	10	steamed	June 30	Deedman's Harbour	-	-
43	Girl	15	7	9	steamed	" "	"	-	-

Table V. Case identifications for persons referred to in Table IV who ate clams without ill effects.

<u>Case No.</u>	<u>Name</u>	<u>Address</u>
33	(not included)	(not included)
34		
35		
36		
37		
38		
39		
40		
41		
42		
43		
44		
45		
46		
47		
48		
49		

Table VI - Questionnaire used to record data on persons who ate toxic clams in 1957.

No.

Case History Paralytic Shellfish Poisoning

Name Date of Illness

Address Time of Onset o'clock

Sex Age Weight . . Duration of Illness
(hours or days)

General Health Date Illness Reported

To Whom Reported How? (Interview etc.)

Source of Shellfish Kind of Shellfish Eaten

Other Food or Drink Taken

Preparation of Shellfish (raw, steamed, etc.)

How Much Shellfish Broth or Bouillion Taken?

Number of Shellfish Eaten Time Date

Toxicity of Raw Shellfish m.u./100g. Estimated Dosage . . . m.u.

Symptoms (Underline symptoms observed and indicate by number 1,2,3,etc., the order in which they appeared.)

Lips	Difficulty with Breathing
Numbness of Face	Standing up
Difficulty with Speech	Difficulty with Sitting up
Fingers	Feeling of Stomach Sickness
Numbness of Toes	Vomiting
Dizziness or Giddiness	Headache
Arms	Backache
Numbness of Legs	Other Symptoms

Any Other Information or Remarks

Table VII. Comparison of 1945 and 1957 epidemics of shellfish poisonings in the Fundy area.

<u>Feature compared</u>	<u>1945 Epidemic</u>	<u>1957 Epidemic</u>
Total number of humans poisoned	26	32
Number of severe poisonings	14	13
Number of deaths	0	0
Areas involved	Nova Scotia & New Brunswick	New Brunswick only
Toxicity of clams chiefly involved in poisonings	18,000	17,800
Minimum score of clams causing illness	6,700	9,850
Date of outbreak	August 16	June 22-23
Water temperatures	Sudden rise preceded outbreak	do
Frequency of causative agent (Dinoflagellate - <u>Gonyaulax tamerensis</u>)	Abundant in plankton	do

A P P E N D I X E

**THE PROBLEM OF UTILIZATION OF CLAM RESOURCES IN ALASKA
DUE TO THE PRESENCE OF PARALYTIC SHELLFISH POISON**

by

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SUMMARY OF CLAM RESOURCES IN ALASKA

In order to fully appreciate the problem of utilization of the clam resources in Alaska, some comprehension of the distances involved is necessary. Therefore, I would like to compare the Alaskan coast line which is productive of shellfish to distances in the United States proper. Figure 1 gives some indication of the comparative distances involved when one considers the productive shellfish coasts of the U.S.A. and the southeast, central and southwestern coast of Alaska. Alaska, by area, equals approximately 1/5 the area of the United States while its coast line equals nearly half that of the United States. However, the coast line of Alaska, amounting to 22,000 miles, is not as accessible and much of it cannot be reached by convenient transportation.

In southeastern Alaska, the productive clam beds are located in an area of islands and narrow passageways. Turbulent tidal currents and hazards of fog and adverse weather make navigation difficult in this area. This portion is separated by a rather long exposed section of coast in the Gulf of Alaska from another shellfish producing area in the vicinity of Cordova which is in the area normally referred to as central Alaska. Farther to the west are the shellfish producing areas adjacent to the Kenai Peninsula, the Alaska Peninsula, Kodiak Island-Shumagin Island section.

Access to most of these shellfish areas by road is almost impossible. Communication is almost entirely by radio or by plane. Each area must therefore be dealt with as a separate problem and that seems to be the picture of development. It is obvious, from the map that a distance of 150 to 300 miles is not significant on the distances shown on the map. Wide separation of producing areas necessitates decentralization of operational control with regard to shellfish sanitation.

The magnitude of the problem is further clarified by knowledge of the distribution of the clam beds along the Alaskan coast. An attempt has been made to show this simply in Figure 2. The southeast coast section has a rather widely and perhaps thinly dispersed series of clam beds, both butter clams and razors. The distribution of cockles is not clearly defined in this area. Moving to the westward near the central portion of the Gulf Coast, we note that a major producing area for razor clams is in the Cordova area where there is also a large quantity of cockles. Moving further to the west, we see a distribution pattern of razor clams at the entrance to the Cook Inlet area, down the Alaskan Peninsula and a series of smaller beds of probable commercial value around Kodiak Island. The distribution of razor clams along the Alaska Peninsula and the Aleutian Chain continues intermittently and with varying degrees of abundance. Some small commercial operations are possible, if the ready access to market can be made available. Butter

clams occur on the Kenai Peninsula, particularly in the Kachemak Bay area, around Kodiak Island, along the south coast of the Alaska Peninsula and also along the Aleutian Chain. The number of commercially significant beds on the Alaska Peninsula and the Aleutian Chain has not been completely established; however, there are known beds considered sufficient to support small commercial activities.

Clam processing plants have been in operation for many years on the southeast, central coast and to a limited extent in the southwestern coast in the vicinity of Kodiak Island. Location of these plants and their main products are indicated in Figure 3 in tabular form. It should be noted that processing activities decreased sharply in southeast Alaska after 1946, when Food and Drug restrictions regarding toxicity of clams made commercial operations rather uncertain. The actual harvesting at the present time is limited chiefly to razor clams with some production in clam nectar and in frozen cockles. The note in 1953 indicates that fresh frozen butter clams are processed for local consumption in Southeast Alaska.

SUMMARY OF TOXICITY CONDITIONS

In 1946 when the first official restrictions were placed on the clam products due to toxicity, the Fisheries Products Laboratory in Ketchikan instituted an active local study of the situation in southeast Alaska. A series of spot-checks and local surveys on clam beaches were conducted in 1946 to 1951. There are several published and unpublished reports and papers on the work accomplished by the staff of the Fisheries Products Lab. An attempt was made to compare beaches and portions of beaches with respect to the behavior of toxicity. The results of the studies are best covered by the reports of the Fisheries Products Lab themselves, however, in Figure 4a and 4b we have summarized very briefly the extent of sampling indicating the numbers of positive samples taken. A positive sample is used here to designate any one of approximately two dozen clams which is found to contain toxicity over the acceptable limit of 400 mouse units.

The work by the Fisheries Products Lab of Ketchikan was confined almost entirely to southeastern Alaska. Interest in further study of the problem of shellfish toxicity developed in 1954 through discussion between the staffs of the Arctic Health Research Center and the Fisheries Products Lab. Further stimulus for expanding the study was provided through outbreaks or indications of shellfish poisoning which occurred along the southwestern Alaska coast. A project concerning study of shellfish toxin was initiated by the Arctic Health Research Center in May 1954 and carried on until September of that year continuing the following year, 1955. The major areas covered by the AHRC studies are indicated in Figure 5. Briefly, the major difficulty encountered was that of gaining access to portions of the coast line to insure sufficiently frequent sampling of the clam beds.

During the summer of 1954, for example, we were able to sample available clam beds only through the assistance of the Fish & Wildlife Service who took us along on their salmon control flights. This resulted in sporadic and haphazard sampling and did not always enable the staff to visit the clam beds at optimum tides. The following year a more definite schedule was followed by visiting specific clam beds every two weeks on the optimum tides by charter aircraft, plus random sampling which was done along the coast by taking passage on a monthly mail boat. Variation in toxicity was found, and Figure 5 indicates those areas where highest toxicity, over 400 mouse units, were located. More frequent sampling in areas where toxicity of less than 400 mouse units is indicated would probably have shown much higher concentrations.

One interesting hypothesis developed in 1954, and unsuccessfully retested in 1955, was that shellfish paralytic toxin occurred more frequently in passages where the ocean currents from the open sea flowed at frequent regular intervals. Shellfish in the waters of passages are exposed to the plankton from the open sea more frequently than the shellfish in the waters of quiet sheltered bays.

POSSIBLE OPERATIONAL PROCEDURES

The basic problem with which we are concerned is that of establishing a more efficient operational procedure for determining the suitability of Alaskan clams for public consumption with regard to paralytic shellfish poison.

Because of the distances involved, the difficulties in communications and lack of land transportation facilities, it would seem desirable that actual determination of toxicity be carried on at the site of the digging operations or clam processing plants. This decentralization of control would enable plant owners to operate at more reasonable costs and with greater flexibility and less risk.

Tests now available would probably have to be conducted at the processing plant. However, samples of clams for testing could be collected in advance of the actual harvesting schedule. This would not guarantee that the clam bed did not develop toxicity between the advanced sampling time and the actual harvesting operation but would certainly reduce the possibilities. The toxicity test would have to be approved and standardized by the Alaska Department of Health, and training courses for plant technicians would have to be instituted and conducted periodically to insure adequate testing.

Test procedures which are presently available are (a) bio-assay mouse test, and (b) the chemical test discussed earlier at this conference. Whether either or both of these tests can be adequately applied at the processing plant has not been determined. However, unless we can revise one or both of these or devise some new technique

for use in the plants, it does not seem to us that it is practical to harvest these clams along the Alaskan coast line in view of the incidence of paralytic toxicity that has been occurring in recent years. Dissatisfaction with a test procedure or accuracy, implies that more research is needed.

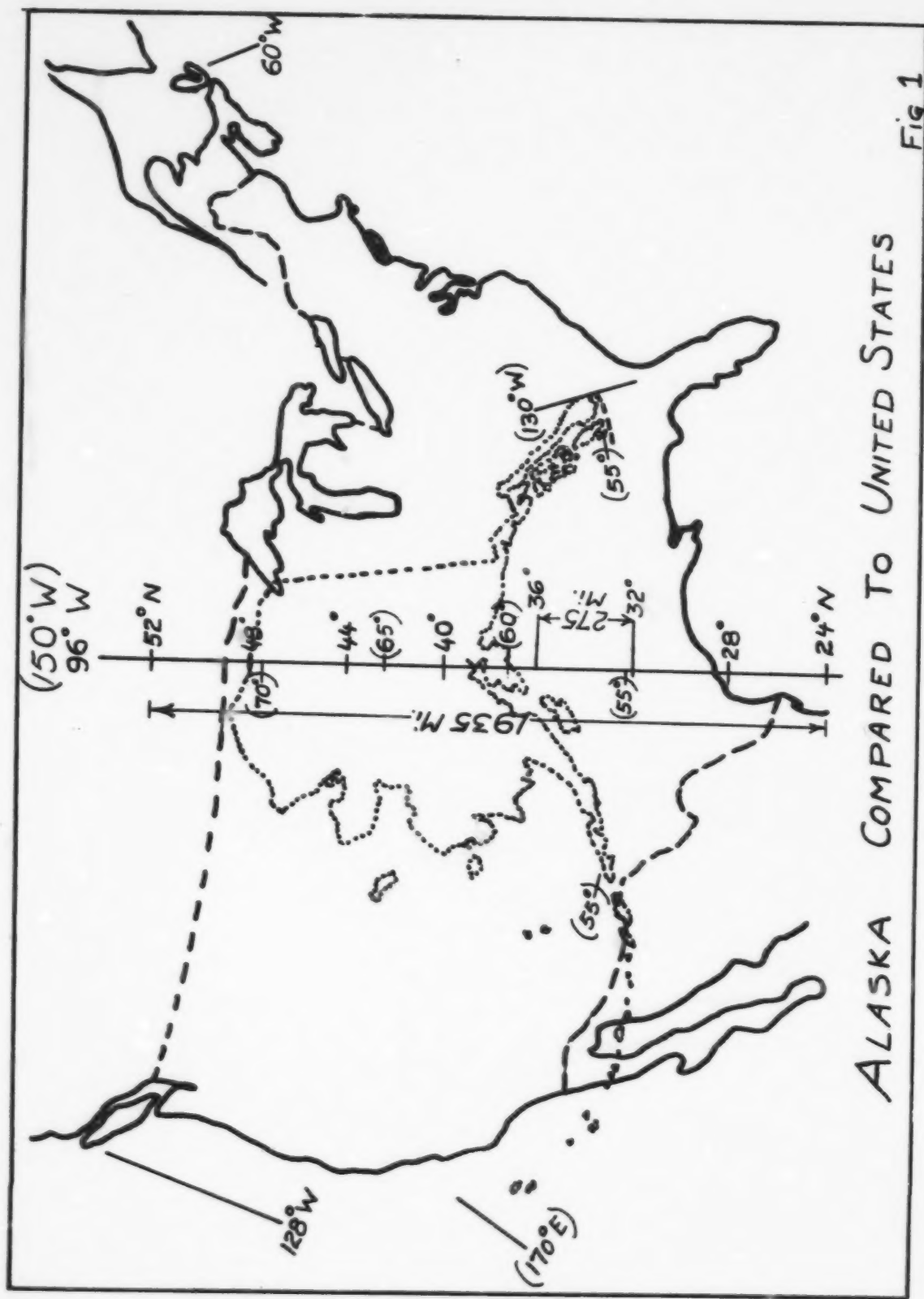
SUMMARY OF RESEARCH POSSIBILITIES IN SHELLFISH PARALYTIC TOXICITY

Discussions among the staff of the Arctic Health Research Center have indicated four lines of research we think could be profitably followed within the next few years. These include:

1. Studies on species selectivity of paralytic toxin, both under conditions of natural assimilation and under experimental conditions where purified toxin is dispersed in water.
2. Studies on the concentration of toxin in various organs of shellfish by species. This would actually be a continuation of previous work on butter clams and could be extended to mussels, razor clams, cockles and deep water species.
3. A basic study should be initiated on a long-range basis to develop a toxin antidote or neutralizer for use either on victims of the toxin or on shellfish which contain the toxin.
4. Additional basic studies should be made on simple field tests for toxicity, such as, neuro-toxin effects on naturally occurring nerve fibers or muscle, such as leech tissue.
5. Studies on the metabolic processes that occur in shellfish tissue when associated with the toxin to determine the process of accumulation in selected organs of the shellfish.

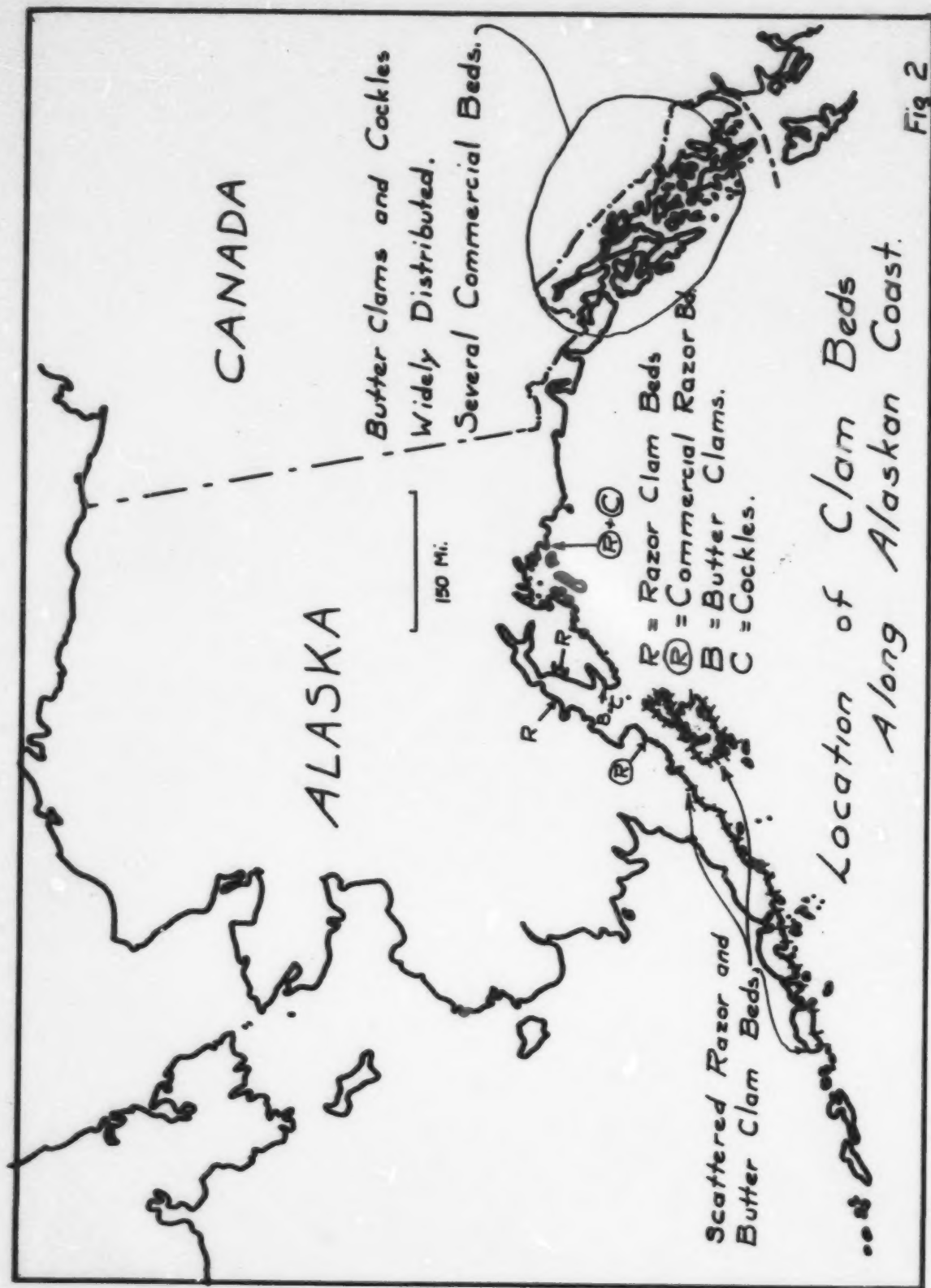
GENERAL CONCLUSIONS

Alaska now has a moderate shellfish potential in the clam products industry. The industry has been rather severely restricted due to the presence of paralytic shellfish poison, which renders the natural product unsuitable for public consumption. If the continued decline in salmon production continues, greater emphasis will be placed on other marine products of Alaska, such as the clam resources. It would seem advisable to develop an operational system or procedure to decentralize the quality control for toxicity content to the clam products processing plants themselves through the media of simplified field tests which have been previously standardized by the Alaska Department of Health authorities. Final control of the product would remain consistent with present procedures of the Food and Drug Administration. Basic research on factors leading to a better understanding of the problem are indicated.



ALASKA COMPARED TO UNITED STATES

Fig 1



CLAM PROCESSING PLANTS**

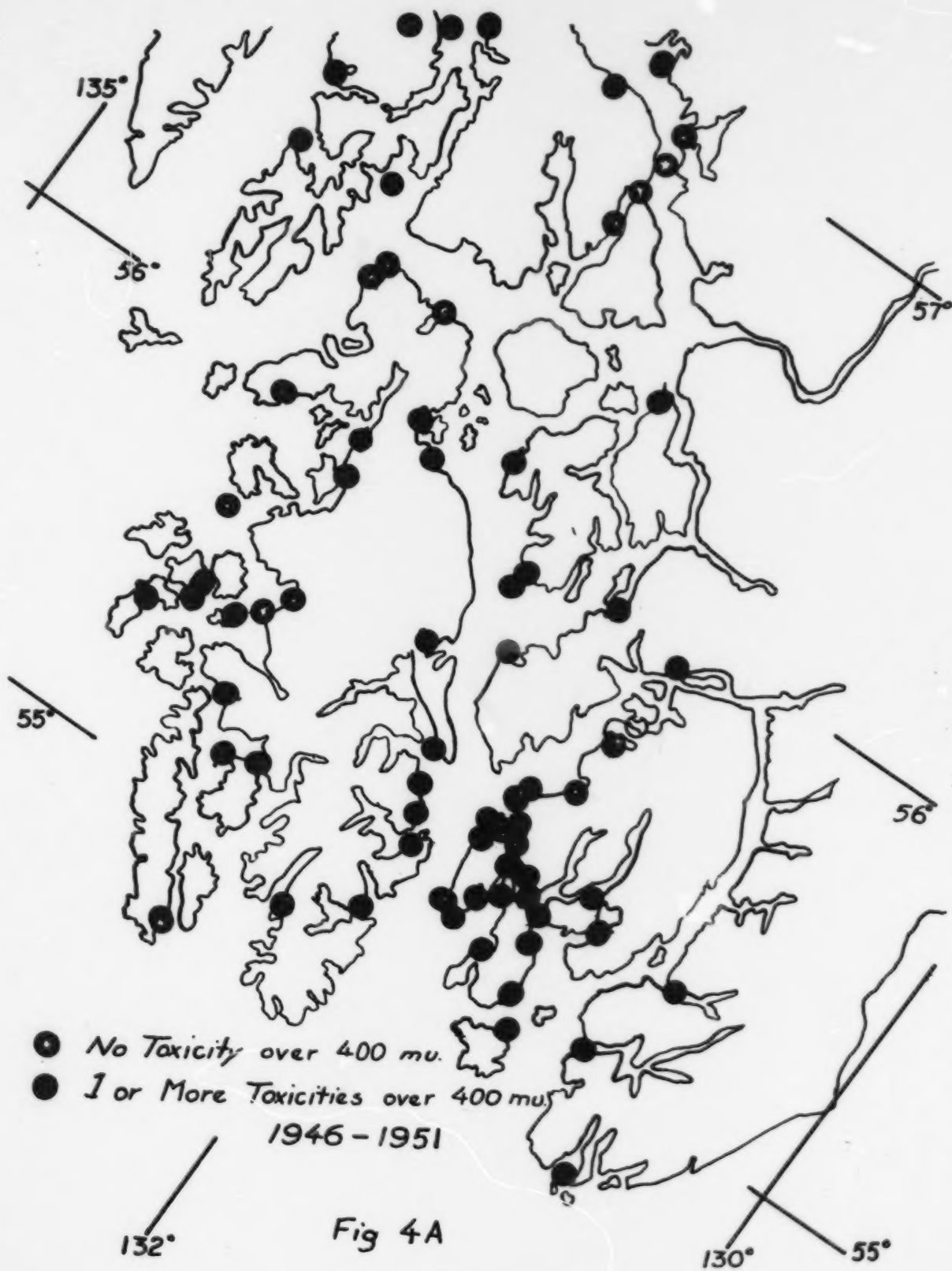
YEAR	S.E. ALASKA	CENTRAL ALASKA	TOTAL	PER CENT RAZOR CLAMS	NO. ENGAGED IN OTHER SEAFOODS
1945	6*	15*	20	50+	No info.
1946	5	15	20	72	No info.
1947	2	18	20	98	11
1953	1	9	10	Bulk***	6
1957	1	10	11	Bulk	3

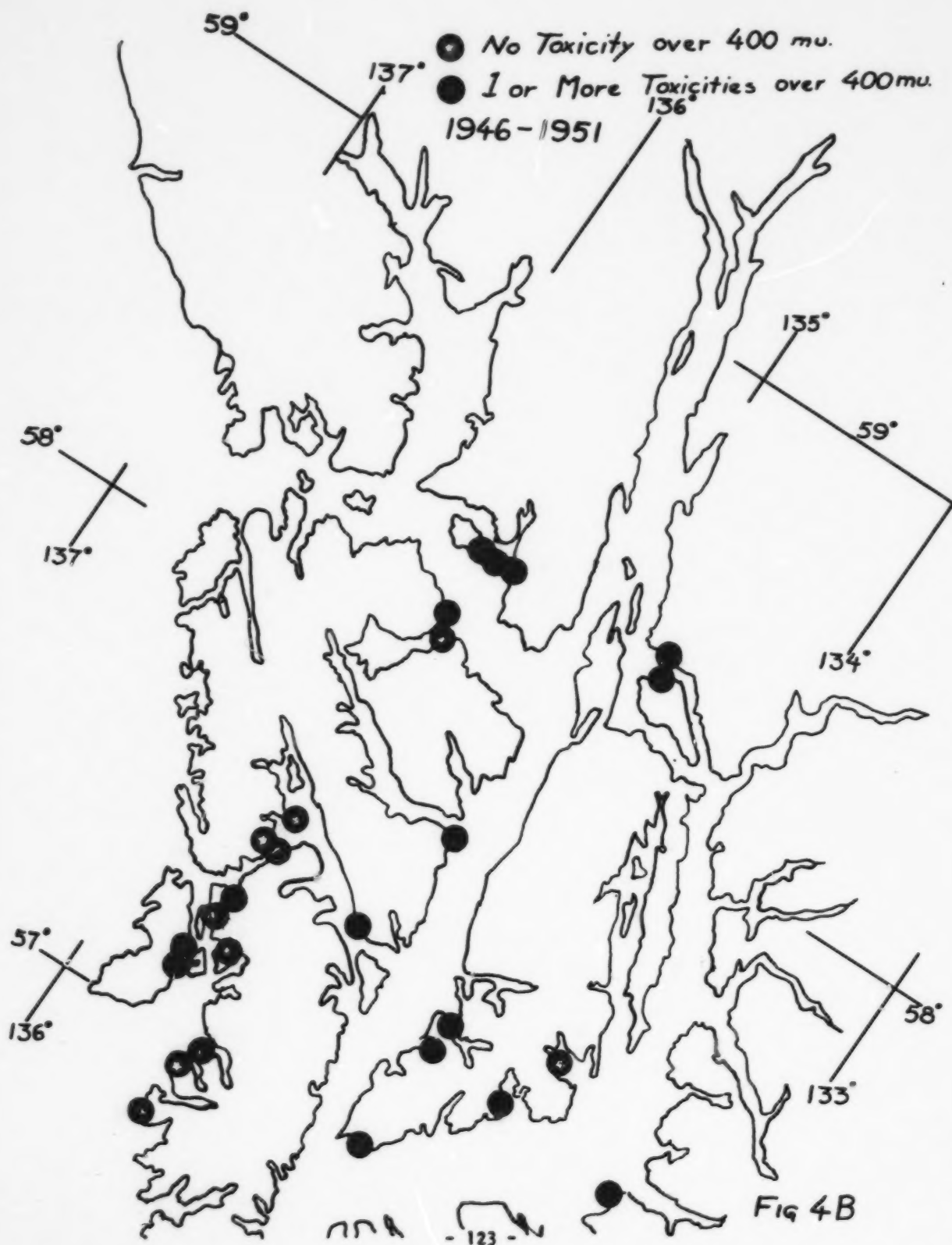
* One operator in both districts.

** Statistical Digest Reports, U. S. Fish & Wildlife Service, Dept. of Interior.

*** Increase in frozen butter clams for local consumption.

Fig. 3





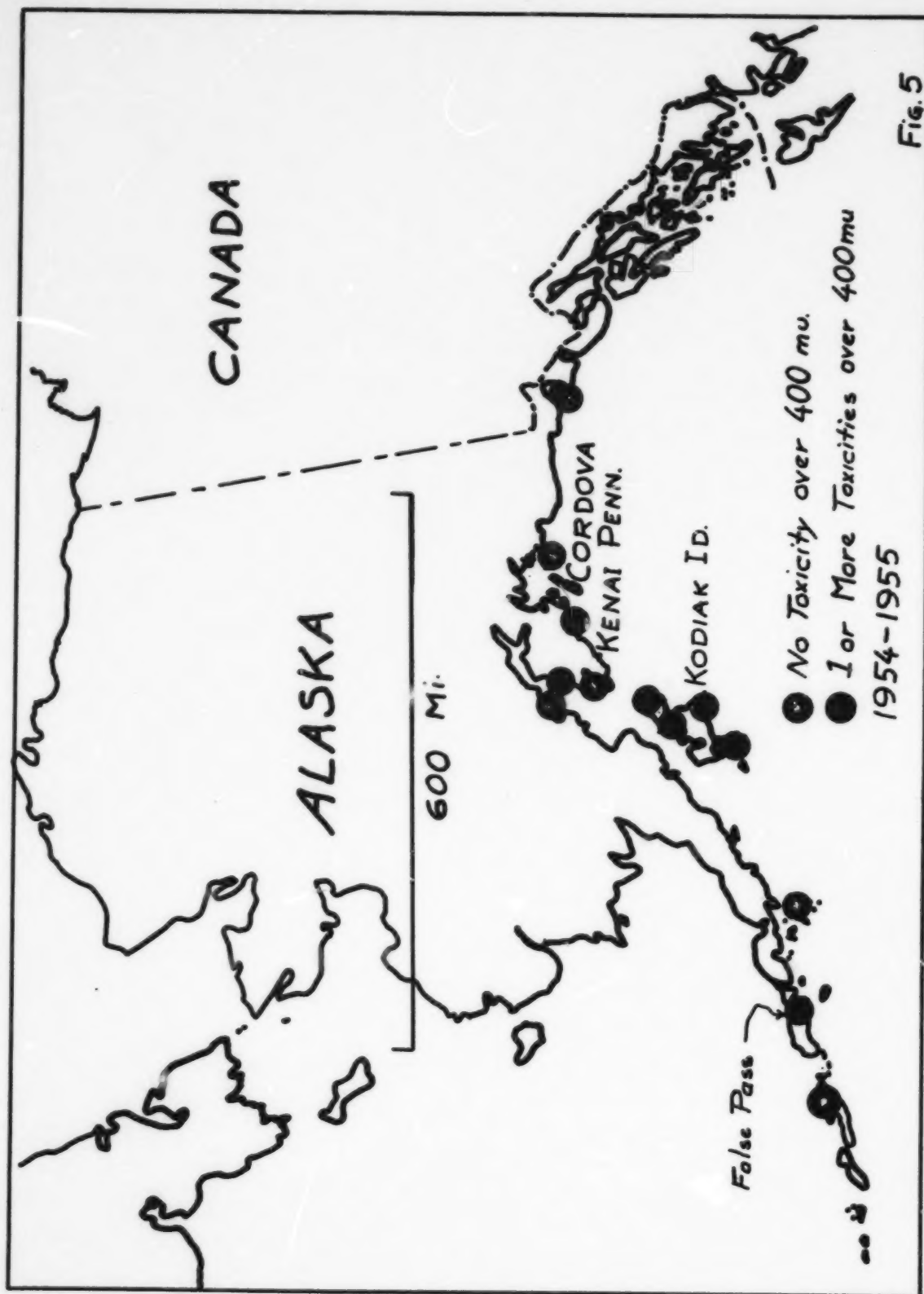


Fig. 5



APPENDIX F

ATTENDANCE AT 1957 CONFERENCE ON SHELLFISH TOXICOLOGY

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